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MAY 1952
VOLUME 53 NUMBER 5

Published Monthly by
AMERICAN MEDICAL ASSOCIATION
535 NORTH DEARBORN STREET • CHICAGO 10, ILLINOIS

Entered as Second Class Matter Jan. 20, 1926, at the Postoffice at Chicago, Under the Act of March 3, 1879. Annual Subscription, $8.00

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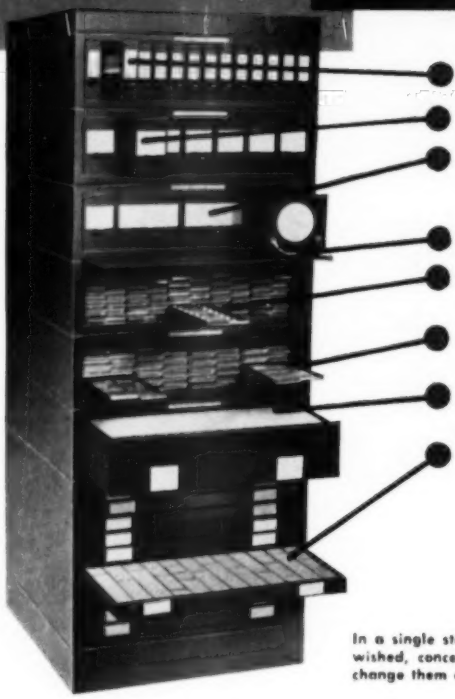

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*Bronstein, M. R., and Witkind, E.: Am. J. Med. Sci. *222*, 677, Dec., 1951.

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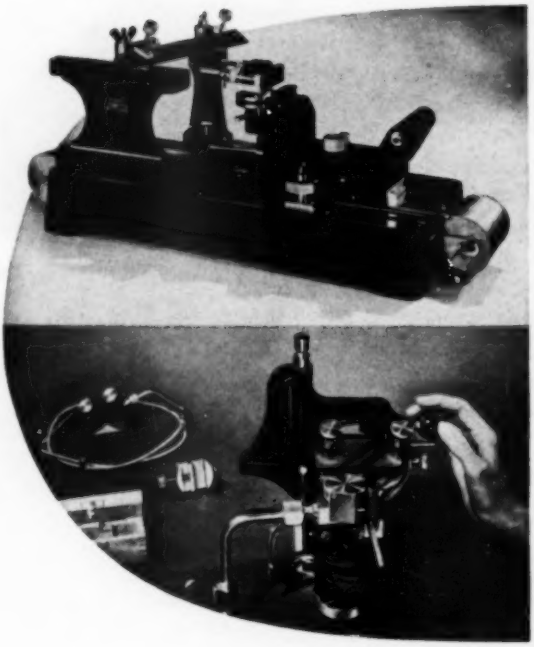

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# SEQUENCE OF CELLULAR RESPONSES TO INJURY IN MICE EXPOSED TO 1,000 R TOTAL-BODY X-RADIATION

LIEUTENANT (JG) JACK BARROW (MCR), U. S. N. R.
AND
COMMANDER JOHN L. TULLIS (MC), U. S. N.

THE EARLY effects of a single total-body exposure to ionizing radiation have been described by many authors from the time of the observations of Heineke,[1] in 1905, to the studies stimulated by the explosion of atomic bombs and the appearance of other possible sources of total-body exposure to ionizing radiation, such as the cyclotron, the atomic pile, and the industrial x-ray tube.

Many of the studies in Bloom's report [2] deal with small groups of animals and with specimens obtained at relatively long intervals following irradiation, so that the sequence of pathologic events is not always portrayed in unbroken progression. Accordingly, a study of a large group of animals from which specimens were taken at short intervals seemed worth while, in order to obtain accurate information as to the rate of appearance and progress of postirradiation pathologic changes.

## MATERIALS AND METHODS

White adult male mice of the National Institutes of Health hybrid strain, weighing 15 to 18 gm. each, were used in the study. Ten served as controls, being subjected to the same caging, transportation, and handling as the irradiated ones. All were fed a commercial food [3] and kept in clean, well-ventilated wire cages in air-conditioned rooms.

The General Electric industrial x-ray tube at the Naval Gun Factory, Washington, D. C., was used as a source of ionizing radiation. Groups of 150 to 200 mice were exposed to the angular beam of x-rays at 1-meter distance from the tube, the technique for simultaneous exposure of large numbers of animals [4] being used. The physical characteristics of the dose of radiation were 1,000 kv., 3 ma., no added filter, half-value layer of 2.2 mm. of lead, 31.23 roentgens (r) per minute measured in air, and 35.1 minutes' exposure.[5] An absolute lethal dose of 1,100 r was used in order to ensure death and thus increase the likelihood of uniform response in the tissues of all animals.

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From the Naval Medical Research Institute (Project NM 007 039, Report No. 23, 1949) Bethesda, Md.

The opinions or assertions contained herein are the private ones of the writers and are not to be construed as official or reflecting the views of the Navy Department or the naval service at large.

1. Heineke, H.: Mitt. Grenzgeb. Med. u. Chir. **14**:21, 1905.

2. Histopathology of Irradiation from External and Internal Sources, edited by W. Bloom, New York, McGraw-Hill Book Company, Inc., 1948.

3. This was purina® fox chow, made by the Ralston Purina Company, St. Louis.

4. Chapman, W. H.; Sipe, C. R.; Eltzholtz, D. C.; Cronkite, E. P.; Lawrence, G. H., and Chambers, F. W. Jr.: Naval Medical Research Institute, Project NM 007 039, Report No. 14, 1948.

5. Tullis, J. L.; Tessmer, C. F.; Cronkite, E. P., and Chambers, F. W., Jr.: Radiology **52**:396, 1949.

Beginning at one hour after irradiation and continuing at hourly intervals up to 88 hours, mice were killed, 10 at a time, with carbon dioxide. Autopsies were made immediately after deaths. All animals found dead in the cages were discarded. Sections of spleen, mesentery, large and small intestines, sternum, testes, liver, and lung were fixed for four hours in Zenker-formol solution, washed for six hours in tap water, and transferred to 80% alcohol. The sections were embedded in paraffin so that one block contained all the tissue specimens from one animal, cut at 6μ, and stained with hematoxylin and eosin. The sternum was not decalcified. Representative tissue sections from the control and experimental mice were also stained according to Giemsa's method.

## RESULTS

Observations of the spleen, lymphoid tissue, bone marrow, the intestine, and the testes will be described in detail. The remaining two organs studied, namely, the liver and the lung, failed to show relevant changes. Single or scattered foci of

*A Summary of the Early Lesions of Acute Radiation Illness in Various Organs of Mice in Order of Increasing Radioresistance.*

| Organ | Major Changes | Minor Changes | Regeneration | Resistant Elements |
|---|---|---|---|---|
| Lymph nodes | Almost complete destruction of lymphocytes | Edema, ecchymosis | Moderate by 75 hr. | Reticuloendothelial cells, plasmacytes |
| Spleen | Destruction of lymphocytes of white pulp and erythroblasts and myeloblasts of red pulp | Erythrocytophagia, increase of blood pigment | Lymphocytopoiesis returns, slight return of myelopoiesis, by 78 hr. | Reticuloendothelial cells, plasmacytes |
| Bone marrow | Almost complete destruction of erythroblastic and myeloblastic tissue | Dilated sinusoids, "ground glass" matrix erythrocytophagia | Slight return of myelopoiesis, by 78 hr. | Reticuloendothelial cells, fat, endothelial cells |
| Testis | Complete destruction of spermatogonia | Moderate destruction of spermatocytes, spermatids, spermia | None seen | Sertoli cells, interstitial cells, prespermatogonia |
| Small intestine | Extensive necrosis of crypt epithelium | Edema of lamina propria | Rapid, almost complete in 80 hr. | Surface epithelium, muscle |
| Large intestine | Moderate necrosis of epithelium at bases of glands | Edema of lamina propria | Rapid, complete by 80 hr. | Mucous cells, surface epithelium, muscle |
| Lung | None | Destruction of lymphoid aggregates about bronchi | ..... | Total lung structure |
| Liver | None | None | ..... | Total liver structure |

degeneration and necrosis of the hepatic parenchyma, infiltrated by heterophiles, eosinophiles, and large and small mononuclear cells, with and without hemorrhage, were noted in the livers of both control and experimental animals, but were infrequent, and were attributed to invading intestinal parasites, with which these mice were uniformly infested. These lesions were not influenced by ionizing radiations.

*Spleen.*—The white pulp in the spleens of the control mice presented lymphoid centers in various stages of activity. The germinal centers contained slight but definite amounts of lymphocytic debris, both free and engulfed in macrophages. This debris was always less in amount than that seen in the spleens of animals immediately after irradiation and probably resulted from the handling, caging, and transportation to which these animals were subjected.

In the irradiated mice the formation of nuclear vacuoles was common in the early hours. The changes characteristic of irreversible cellular degeneration and death,

namely, pyknosis, karyorrhexis, and karyolysis, were readily apparent in the earliest specimens examined. Degeneration and diminution of the number of lymphoid elements in the spleen progressed through the 88-hour observation period. Cellular debris continued to increase to a maximum at four hours, remained at a peak until about nine hours, and gradually declined until, at 24 hours, several spleens were largely free of debris. By 30 hours most of it was cleared.

Phagocytosis of debris, already actively taking place in the one-hour specimens, proceeded rapidly until, by 21 hours, most of the debris which remained was intracellular, the greater portion having already been digested. Five hours after irradiation the structure of the white pulp was so obscured that such formations as germinal centers and reaction centers of Hellman could hardly be identified as such. By 20 hours large areas in the germinal centers had been stripped of lymphocytes, and only masses of large reticular cells remained. We observed that a few groups of these had coalesced to form a syncytium such as was described by Henshaw.[6] Subsequently these masses of reticular cells assumed the appearance of fibroblasts. Concomitant diminution of the size of the white pulp centers took place until, at 80 hours and beyond, the white pulp was represented by small dense islands of reticular cells and plasmacytes.

Mitotic figures were numerous throughout the white pulp of the control spleens but quickly disappeared after irradiation, as no mitosis was observed in the white pulp from one through four hours. The return of mitosis by the end of five hours was indicated by observation of at least one and often several mitotic figures in the white pulp in seven out of the nine spleens available for that hour. Mitoses were present in all specimens thereafter and became numerous in the period from 10 to 36 hours, although the numbers present at this time never equaled or exceeded the normal rate. Thereafter mitotic activity fell off until only a rare figure could be detected in each of the mouse spleens of 70 hours and beyond. Abnormal mitoses, with tripolar or multipolar origin or lagging or clumped chromosomes, were not infrequent five hours and later after irradiation.

Evidence that lymphocytes were regenerated in the irradiated mice was slight. Our techniques were not sufficiently critical either to confirm or deny Bloom's belief that some lymphocytes develop from reticular cells as early as three hours after irradiation.[2] It should be emphasized, however, that no morphologic changes were noted in the reticuloendothelial cells at any time in any of the irradiated mice, and these cells apparently serve as the origin of most of the regenerated lymphocytes. By 75 hours moderate numbers of small lymphocytes had reappeared, densely packed, in the lymph sinusoids in the region of the central arterioles of the spleen.

The observation that the white pulp of the spleen, as well as centers of lymphoid tissue in the lymph nodes and in the intestine, is invaded by mature members of the granulocytic series after irradiation moved Lacassagne and Lavedan[7] to conclude that the polymorphonuclear leukocytes took part in the process of phagocytosis of debris. In this study, heterophiles and eosinophiles could be observed within the pulp of the spleen in numbers as early as eight hours, at a maximum at about 28 to 30 hours, and persisting as long as 55 hours. However, at no time were these cells observed to contain phagocytosed debris.

6. Henshaw, P. S.: J. Nat. Cancer Inst. **4**:477, 1944.

7. Lacassagne, A., and Lavedan, J.: Paris méd. **51**:97, 1924.

Plasmacytes can be observed in rather small numbers in the spleen of the normal mouse, usually singly or in clumps peripherally around germinal centers or along trabeculae. Increase in the number of plasmacytes was apparent four hours after irradiation, and the proportion of plasmacytes steadily increased thereafter throughout the entire series. After 80 hours these cells were arranged in large clumps and sheets, and seemed to make up the major portion of some of the small islands of white pulp which remained. We did not detect degenerative changes in plasmacytes at any time following total-body irradiation. It appears likely that some of the increase in the number of plasma cells is due to shrinkage of the splenic tissues, particularly of the white pulp, and to disappearance of the cells of the lymphocytic and hematopoietic series. However, the finding of an increased number of plasmacytes at an early time, before shrinkage of tissues is marked, makes it seem likely that at least some of the increase is due to their having developed from other forms, probably lymphocytes, as suggested by Bloom.[8]

A considerable part of the red pulp of the spleen of the normal mouse is composed of hematopoietic tissue. This is heavily distributed principally in the subcapsular region. Both the erythropoietic and the granulocytic, or myelopoietic, series are represented there, and megakaryocytes appear in profusion. The response of the hematopoietic tissue of the spleen to 1,100 r total-body irradiation was well established in the one-hour specimens, which showed an absence of mitoses, pyknosis, and karyorrhexis of cells of the erythropoietic series and already a diminution in number of early erythroblasts. Mitotic figures were not observed in the red pulp of the spleen until eight hours after irradiation, in contrast to their earlier reappearance in the white pulp. Debris in the red pulp during the early hours seemed to arise from the cells of the erythropoietic series, and by six hours destruction of this series was striking. A few normoblasts were seen as late as 60 hours after exposure, but the more immature elements of the red cell series were largely gone by 10 hours. No sign of regeneration of the erythropoietic cells was ever seen in the spleen. The myelopoietic cells degenerated at a somewhat slower rate. Several myeloblasts and many of the myelocytes with doughnut-shaped nuclei, frequently seen in mouse hematopoietic tissue, were prominent in the 6- to 10-hour specimens, and the latter form persisted in fair numbers for as long as 30 hours. In the eight-hour sections, and subsequent ones, very large myelocytes with multilobated nuclei were noted, and in sections taken from 16 to 36 hours these megamyelocytes were represented by numbers of large hypersegmented heterophiles. Increase in number of mature members of the polymorphonuclear series was apparent by eight hours; it became prominent by 20 hours and remained so through the 50-hour specimens, after which the numbers of heterophiles and eosinophiles steadily declined. A few, however, could always be discovered in all sections through the 88-hour specimens. A few clumps of myelocytes with doughnut-shaped nuclei seen under the capsule or along the trabeculae at 78 hours or after suggested abortive regeneration.

The megakaryocytes of the spleen were not affected until some 50 hours after irradiation, when a decrease in number became apparent. This diminution continued throughout the remainder of the series, but even at 80 hours and beyond several were still detectible. In the later hours some of the megakaryocytes had shrunken

8. Bloom, W.: Handbook of Hematology, edited by H. Downey, New York, Paul B. Hoeber, Inc. (Medical Book Department of Harper & Brothers), 1938; footnote 2.

nuclei with fewer lobes and a diminished amount of cytoplasm, but occasional megakaryocytes bearing this configuration would be seen in normal mouse spleens, and, conversely, several morphologically normal megakaryocytes could be viewed in all the irradiated spleens. It cannot be determined from this study whether the changes noted above represented a degeneration, the result of irradiation, or a process of attrition without replacement. In any case the effect of a lethal dose of ionizing radiation affected the megakaryocytic series much less than the other members of the hematopoietic series.

Another interesting process noted in the red pulp after irradiation was that of erythrophagocytosis or erythrocytophagia. This phenomenon occurs to a minor extent in normal mouse spleen, but was observed to occur more often eight hours after irradiation and to increase to a maximum in 60 to 70 hours, when many tissue macrophages and other reticuloendothelial cells in the sinusoids of the red pulp and around the periphery of the white pulp were packed with red cells which appeared morphologically normal or were in various stages of degeneration. By 80 hours a number of macrophages contained large amounts of yellow, granular pigment, assumed to be hemosiderin. Control mouse spleens contained a small amount of the same intracellular and extracellular pigment, but that in the irradiated spleens exceeded this in amount after 60 hours had elapsed.

As a result of these degenerative processes following total-body irradiation, the mouse spleen lost its thick subcapsular layer of hematopoietic tissue in 36 hours, leaving the capsule and trabeculae bare. By 88 hours the spleen consisted of small islands of pulp almost devoid of lymphocytes, and shrunken into small clumps of reticular cells and plasmacytes. These islands were surrounded by dilated sinusoids packed with red cells, and a reticular framework in which were enmeshed a moderate but increased amount of extracellular pigment, diminished numbers of megakaryocytes, macrophages laden with hemosiderin and red cells, and rare, small, scattered islands of regenerating myelocytes.

Analysis of early effects of a lethal dose of ionizing radiation on lymph nodes and intestinal lymphoid tissue in this study revealed a progression of events strikingly parallel to those taking place in the white pulp of the spleen. This is in accord with the findings of Henshaw,[8] who studied mice, and Stearner, Simmons, and Jacobson,[9] who studied rats, and in contrast to the report of Lawrence, Dowdy, and Valentine,[10] who observed that the lymph nodes and intestinal lymphoid tissue underwent more rapid change than did the spleen. While the amount of damage in one site of lymphoid tissue might exceed that in another in the same mouse at a given time, the circumstances were as frequently found to be reversed in another mouse killed at the same hour. This observation will be expanded upon later.

The changing histopathologic aspect of the spleen as estimated at various times after irradiation is shown in Figure 1.

*Lymph Nodes.*—Lymph nodes were available for study from 178 of the 880 experimental mice and from all of the controls. While the number of specimens was smaller than is desirable, the distribution was sufficiently dispersed through the

9. Stearner, S. P.; Simmons, E. L., and Jacobson, L. O.: Atomic Energy Commission, MDDC 1319, 1947.

10. Lawrence, J. S.; Dowdy, A. H., and Valentine, W. N.: Atomic Energy Commission, MDDC 853, 1947.

series to make the study profitable. Most of the nodes were of mesenteric origin. Numbers, too few to allow comparison of the nodes from the different sites, were obtained from the thorax in conjunction with sections of sternum or lungs. These showed the same qualitative changes as those from the peritoneal cavity.

The structure of mouse lymph nodes and the proportion of lymphoid nodules to loose lymphatic tissue are variable, and consequently the effect of ionizing radiation on the general node structure is somewhat difficult to interpret. An additional factor complicating interpretation was the presence in the mesenteric lymph nodes of occasional foci of necrosis about an amorphous, usually basophilic, degenerate mass which was attributed to the parasitic infestation of these mice. It is none the less clear that the radiation-induced pathologic changes in germinal centers of lymph nodes were similar in all respects to those in the white pulp of the spleen; i. e., they were similar in time of onset, rate of progression, and morphology, so that the previous description of the behavior of the splenic white pulp is applicable here. Most of the phenomena seen in the spleen, e. g., abnormal mitosis, syncytial formation of reticular cells, granulocytic invasion, increase in number of plasmacytes, and erythrophagocytosis, were equally apparent in lymph nodes. By 18 to 21 hours the

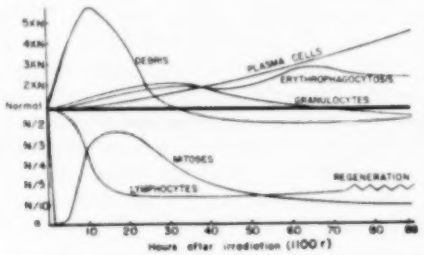


Fig. 1.—Estimated quantitative changes from the normal in the spleen of mice examined 10 at a time at hourly intervals during an 88-hour period following irradiation. Note the rapid cessation of mitotic activity, followed by partial resumption of division; the rapid destruction of lymphocytes, which is paralleled by the accumulation of debris; the decrease in debris as phagocytosis becomes more active; the gradual relative and absolute increase in plasmacytes, and granulocytes; the eventual decrease in granulocytes; the increased phagocytosis of erythrocytes.

germinal centers were almost depleted of intact lymphocytes and the peripheries were made up of cells with large, pale ovoid nuclei and abundant, slightly acidophilic cytoplasm. Edema became prominent. By 80 hours the reticulum of the nodes was the dominant feature, with numbers of plasmacytes and scattered small lymphocytes enmeshed in it.

*Lymphoid Tissue of the Intestines.*—The sequence of pathologic events in Peyer's patches of the ileum and in the solitary lymphoid nodules of the colon, following total-body irradiation, were studied in 295 experimental specimens and in 8 of the 10 controls. As was the case with the lymph nodes, the number was smaller than is desirable, but the distribution among the total number of mice was fairly satisfactory. The normal structure of the lymphoid aggregation was somewhat variable, as sometimes one or two discrete germinal centers could be delineated, whereas at others the lymphoid aggregation was diffuse and lacked a discrete pattern. A certain amount of cellular debris existed in these intestinal lymphoid nodules in the control mice, particularly in the nodules which contained germinal

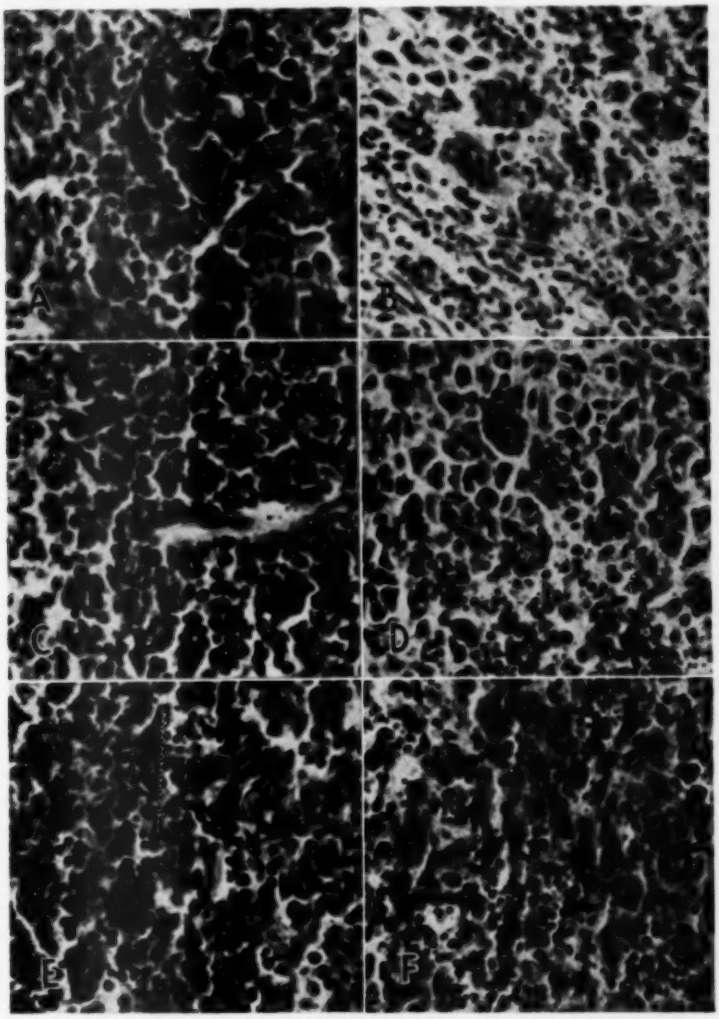


Fig. 2.—*A*, spleen from a mouse one hour after irradiation. Necrosis and fragmentation of lymphocytes are already in progress. The lesions are similar to those shown in *C* and *E*. × 530.

*B*, spleen from a mouse nine hours after irradiation. Necrosis of lymphoid elements is extensive. Many of the nuclear fragments are in phagocytes. Compare with *D* and *F*. × 530.

*C*, mesenteric lymph node one hour after irradiation. Necrosis and fragmentation are about as far advanced as in the spleen (*A*) and the intestine (*E*). × 530.

*D*, mesenteric lymph node nine hours after irradiation. The type and the extent of injury are similar to those shown in *B* and *F*. × 530.

*E*, solitary lymph nodule in the wall of the small intestine of a mouse one hour after irradiation. Compare with *A* and *C*. × 530.

*F*, solitary lymph nodule in the wall of the small intestine of a mouse nine hours after irradiation. Compare with *B* and *D*. × 530.

centers. Radiation-induced pathologic changes in the intestinal lymphoid tissue pursued the pattern described for the splenic white pulp and lymph nodes. In Figure 2, *E* and *F* show representative specimens of intestinal lymphoid tissue. After 55 to 60 hours Peyer's patches shrank to a thin, flattened area of loose fibrous or reticular tissue interspersed with a few small lymphocytes and many plasmacytes. Although a moderate influx of red cells was noted in the germinal centers of all of the lymphoid tissues, hemorrhage of any magnitude was never encountered. We detected no consistent differences in the over-all degree of damage or in the rate at which debris was removed from the lymphoid tissues of the spleen, lymph nodes, or intestinal lymphoid nodules, although not infrequently one animal showed some difference between one site and another. The similarity of histologic pattern in the lymphoid organs is brought out by comparison of *A*, *C*, and *E* and *B*, *D*, and *F* in Figure 2.

*Pulmonary Lymphoid Tissue.*—In passing, it may be noted that while, as was stated previously, the lung itself revealed no significant change consequent to a single lethal dose of total-body irradiation, the "collars" of lymphoid tissue seen about the larger bronchi and pulmonary vessels near the hilus of the mouse lung do show the destruction of lymphocytes and resolution by phagocytosis characteristic of the reaction in lymphoid aggregations elsewhere.

*Bone Marrow.*—Examination of cross sections of the sternal marrow of the control mice revealed them to be densely cellular with a minimal amount of debris, active mitosis, very rare plasmacytes, and a relative preponderance of members of the granulocytic series, including a high per cent of eosinophiles, which was perhaps consequent to the parasitic infestation. After irradiation the mitotic activity disappeared until the eighth hour, when it began to recur in a spotty, irregular fashion. By 14 hours mitoses were plentiful and remained so until 36 hours, thereafter diminishing until none was seen beyond 64 hours. Large multipolar mitoses and other abnormal mitotic figures were frequent. The erythroblasts were affected in one-hour specimens in moderate numbers, as these cells began to show nuclear vacuolation, pyknosis, and karyorrhexis. Debris of this origin was prominent at three hours and reached a maximum by nine hours, at which time most of the erythroblasts were gone.

The normoblasts followed at a slower pace, a few of them remaining discernible in the marrow for as long as 66 hours. Changes in myelocytes became noticeable within eight hours with increase in size and formation of multilobed dense nuclei. These megamyelocytes went on to develop into large, hypersegmented heterophiles and eosinophiles, which probably largely degenerated *in situ* over an extended period. Actual debris resulting from disruption of members of the granulocytic series could be detected around 12 hours, and active degeneration of myelocytes with doughnut-shaped nuclei was visible at 32 hours. However, by this time the mature members of the granulocytic series dominated the field. These heterophiles and eosinophiles, many of which were abnormally large, were plentiful up to 60 hours following irradiation, and a few persisted throughout the entire 88 hours. The megakaryocytes, like those in the red pulp of the spleen, did not appear to undergo morphologic change during the first 48 hours after irradiation. During this time they engulfed various other forms, such as heterophiles, erythrocytes, and cell fragments. Thereafter increasing numbers of the megakaryocytes underwent loss of

shrinkage of their cytoplasm and a reduction in number of nuclear lobes. Some were pyknotic. A decrease of the total number of megakaryocytes in the marrow appeared to be taking place by 70 hours; nevertheless, several remained in the

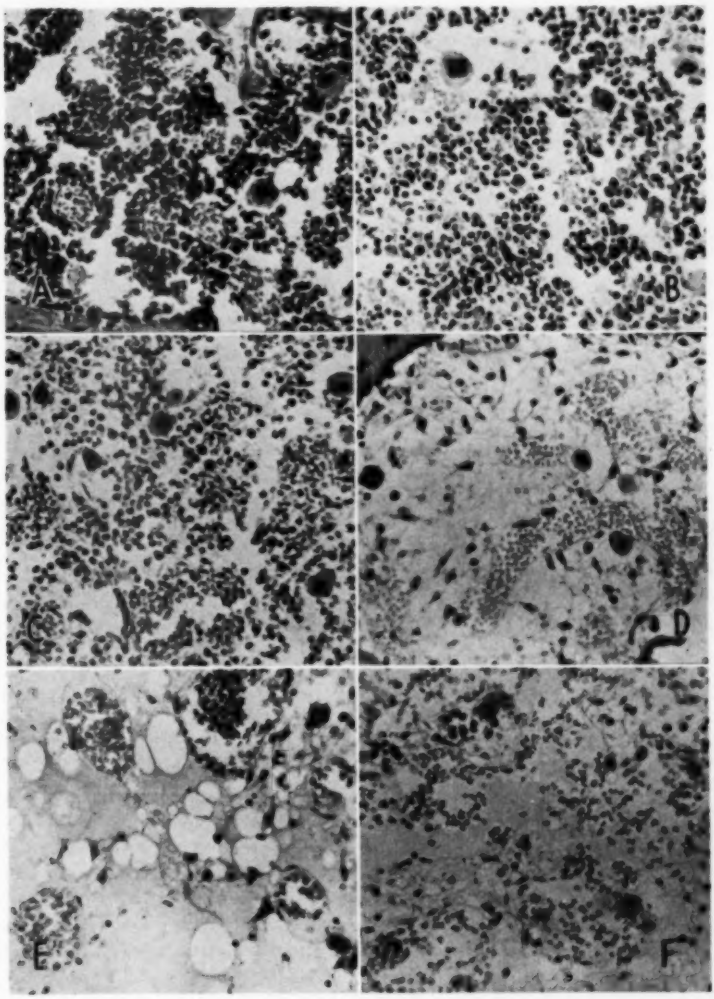


Fig. 3.—Photomicrographs of the sternal marrow of mice 5, 17, 30, 50, 70, and 86 hours, respectively, after irradiation. Note the progressive depletion of cellular elements. × 230.

88-hour sections. A finely granular, amorphous, slightly basophilic ground substance was detectible in spaces between the remaining cells as early as three hours after irradiation, and it steadily increased in amount. Specimens taken at 36 hours and

subsequently showed an increasing amount of space taken up by dilated blood sinusoids, and after 65 hours the proportion of space occupied by the dilated sinusoids or matrix substance varied with the animal. Erythrocytophagia on the part of the reticuloendothelial cells lining the sinusoids became common after 24 hours and continued through the 80-hour specimens, at times assuming striking proportions. Plasmacytes, rarely seen in the control marrows, became progressively common after 45 hours, and were plentiful in all of the late specimens. This increase in the number of plasmacytes in the marrow can hardly be due to shrinkage of the tissue, and we are led to the conclusion that they might represent transitional forms of other cells. The endothelial fibroblasts of the marrow were not observed to undergo morphologic degenerative change.

Regeneration by the erythropoietic elements of the marrow was not observed. However, beginning in the 78-hour specimens, and frequently thereafter, clumps of poorly differentiated cells were observed. Some 87- and 88-hour marrow sections appeared to contain definitely increased numbers of heterophiles and eosinophiles, which, we infer, arose from the poorly differentiated cells, possibly myeloblasts, noted in the earlier sections. Photomicrographs of the progression of pathologic

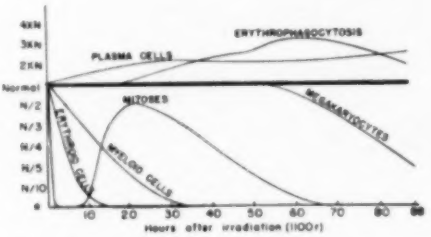


Fig. 4.—Estimated quantitative changes from the normal in the sternal marrow of mice examined 10 at a time at hourly intervals during an 88-hour period following irradiation. Note the relative radioresistance of the developing erythroid and myeloid cells and the megakaryocytes, the rapid cessation of mitosis, with subsequent partial but temporary recovery, and the increase in plasmacytes and erythrophagocytosis.

events in the marrow are presented in Figure 3. An estimate of the sequence of pathologic events in the marrow is presented in Figure 4.

*Testis.*—Evaluation of the early pathologic testicular sequelae following a lethal dose of total-body irradiation is somewhat difficult owing to the natural variability of activity from tubule to tubule in normal mice, to the presence of a few degenerating cells in the normal tubules at all times, and to the difficulty in estimating the over-all numbers of spermatocytes and spermia present.

Bloom [11] has applied the term "prespermatogonia" to the basement membrane cells having ovoid nuclei, and from our observations we would agree as to its applicability. A review of the histologic description of the normal mouse testis provided by Bloom is recommended prior to study of the response of testicular tissue to irradiation. A picture indistinguishable from the normal is shown in Figure 5*A*.

Following a single total-body exposure to 1,100 r of x-rays, the earliest change detectible in this series was a slight increase in the number of vacuolated and

11. Maximow, A. A., and Bloom, W.: Textbook of Histology, Ed. 4, Philadelphia, W. B. Saunders Company, 1942.

karyorrhetic cells near the basement membrane by eight hours. Prior to this time there was not a sufficient number of necrotic cells to be distinguishable from the normal state. Contrary to what was observed in other tissues, no diminution of the number of mitotic figures was detected among the spermatocytes.[11a] Subsequent

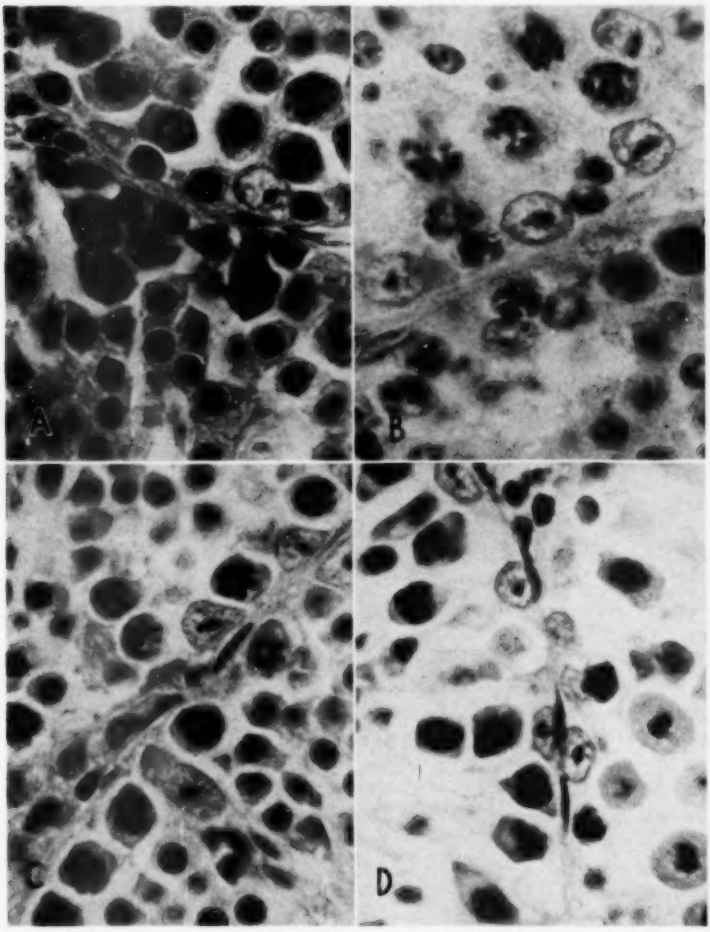


Fig. 5.—Photomicrographs of the testes of mice 3, 37, 52, and 88 hours, respectively, after irradiation. There is progressive depletion of germinal cells. At 88 hours the nuclear patterns are altered and the organ is edematous. × 1,035.

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11a. "Mitosis" is used here as a general term to indicate cell division. Cells in meiosis (spermatocytes) appear to be less sensitive to radiation than cells in normal somatic division (spermatogonia).

to eight hours there was a gradual increase in the number of necrotic cells near the basement membrane, and in the number of giant cells in the lumen, until a peak was reached between 20 and 25 hours. Again this was difficult to evaluate quantitatively, and the amount of early damage was exceedingly slight in the mouse testis, in comparison with that seen in other radiosensitive tissues. Most of the debris was cleared away from the basement membrane by 36 hours (Fig. 5*B*). Concomitantly there was a steady decline in the number of spermatogonia of both types, and it was noted that the spermatogonia with dust-like nuclei disappeared at about 44 hours, whereas a few spermatogonia with crust-like nuclei were detected as late as 60 hours. Thereafter no spermatogonia of any sort were seen. The spermatocytes did not undergo definite diminution in number until after 48 hours, but by 36 hours some degenerative changes could be seen in them, and in the later specimens several spermatocytes had karyolytic or vacuolated nuclei, and the number of spermatocytes was probably reduced (Fig. 5*C*). In later specimens the spermia included an increased number of degenerate or abnormal forms characterized by vacuolated or irregular heads. Pyknosis and bizarre nuclear forms were in the majority by the 88th hour (Fig. 5*D*). The amount of damage seen early seems insufficient to explain fully the diminution of the number of spermatogonia, and it

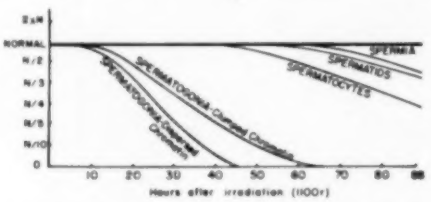


Fig. 6.—Estimated quantitative changes from the normal in the testes of mice examined 10 at a time at hour intervals during an 88-hour period following irradiation. Note the progressive destruction and relative radioresistance of the several germinal cells.

appears possible that some of the spermatogonia went on to mature into the abnormal or degenerating spermatids and spermia noted above. The basement membrane cells were detectible in largely an unchanged state throughout the series, and no qualitative or quantitative changes were ever observed in the Sertoli cells. Figure 6 presents an estimate of the degree of damage sustained by the various germinal elements during the 88-hour observation period following irradiation. The interstitial tissue of the testes likewise underwent very little change. Some degree of edema was noted in specimens from 36 to 60 hours (Fig. 5*C*), but no other significant changes were noted, and no alterations of the interstitial cells of Leydig were observed.

*Intestines.*—Sections of jejunum (several from each mouse), ileum, and large intestine were available for study from most of the control and experimental mice. A small number of necrotic cells with pyknotic or fragmented nuclei were commonly noted in the crypts of Lieberkühn of the control animals. Mitotic activity was great in the crypts of the jejunum and somewhat less in the ileum and in the large intestine. This activity was notably absent in all sections of intestine from one through seven hours following irradiation. However, mitoses reappeared in four of the nine sections of small intestine taken at eight hours and in all of the

eight sections available at nine hours. Mitosis reappeared in two of the nine sections of large intestine available at eight hours, and in seven out of eight of the nine-hour specimens. Thereafter mitotic activity rapidly returned to and remained at the normal level. Changes consisting of vacuolation, pyknosis, and karyorrhexis were

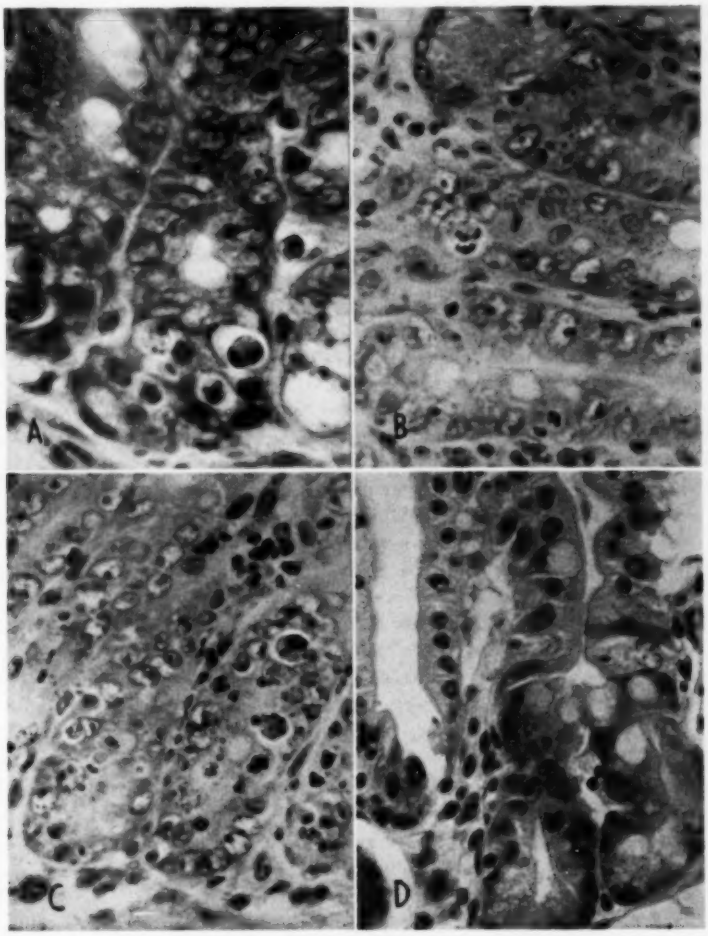


Fig. 7.—Photomicrographs of mucosa of the small intestine of mice 5, 17, 29, and 65 hours, respectively, after irradiation. Necrosis of the epithelium deep in the crypts is increasingly evident through 29 hours, but return to normal is almost complete by the 65th hour. *A*, × 1,035; others, × 530.

noted in increased numbers in the crypts of Lieberkühn in the one-hour specimens, and the amount of damage steadily increased to a maximum at from 5 to 10 hours (Fig. 7*A*) in the small intestine. The amount of damage in the large intestine was

much less, and it was not until two or three hours had elapsed that the number of necrotic cells could definitely be called abnormal. The amount of irreversible cellular damage was never as great as in the small intestine. So far as we were able to determine, the epithelial debris was cleared from the crypts and glands by being extruded into the lumen. This was largely accomplished by 50 hours. Varying amounts of debris were noted in the crypts thereafter, but seldom exceeded the amounts noted in the control sections.

Another phenomenon attendant on a lethal dose of x-rays was the formation of large nuclei with prominent rounded nucleoli and abundant nuclear sap among the crypt cells which survived the immediate cellular destruction. These cells are commonly referred to as possessing "owl's eye" nuclei, and were present in the crypts of the jejunum and ileum and in the deeper portions of the mucous glands of the large intestine, beginning as early as 12 hours after irradiation and frequently seen by 17 hours and later, e. g., in *B* and *C* of Figure 7. Moreover, the cells so affected did not appear to die but gradually moved up the sides of the glands and villi to the tips whence, presumably, they were sloughed in the usual process of attrition. Few cells with "owl's eye" nuclei were seen after 70 hours. In the colon the progress was similar but could not be as easily followed. It is of interest to note that no mucous cells were ever seen to possess "owl's eye" nuclei; only the indifferent cells were so affected.

The cells of Paneth, lying at the bases of the crypts of the small intestine and containing eosinophilic cytoplasmic granules, were not markedly affected by the irradiation (Fig. 7*B* and *D*). Few of them contained pyknotic or karyorrhetic nuclei in the early hours following irradiation, and none possessed the "owl's eye" nucleus. This may be a difference which is more apparent than real and due to the much smaller proportion of Paneth cells in relation to the others.

The regenerative capacity of the epithelium of both the small and the large intestine was striking. No areas of ulceration or denudation were ever seen and by 65 hours (Fig. 7*D*) and thereafter the crypts appeared almost indistinguishable from those of the control mice. The epithelium of the colon returned to a normal appearance even earlier. We were unable to observe clear-cut evidence to suggest that secretion of mucus was increased in the large intestine.

The lamina propria of the intestine also underwent some change following irradiation. During the first several hours, degenerating free lymphocytes could be seen, particularly in the villi. Between 18 and 48 hours the lamina propria was rather heavily infiltrated with heterophiles and eosinophiles; thereafter the lamina propria assumed a hypocellular appearance. However, an occasional eosinophile and several plasmacytes were observed in sections from any period. Beginning at approximately 28 hours and continuing to a maximum from 60 through 70 hours, the lamina propria of the small intestine and to a lesser extent that of the large intestine became edematous. The villi of the jejunum and ileum and the mucosal folds of the large intestine, as a result, took on what Henshaw [6] has aptly referred to as a club-shaped appearance. This subsided rapidly at from 70 to 80 hours, and late 80-hour sections of small and large intestine appeared nearly normal.

*Liver.*—The liver was studied thoroughly, and no significant lesions of parenchyma, ducts, or reticuloendothelial cells were seen. The focal lesions described earlier were assumed to be incidental. It is worth while to note that erythrocyto-

phagia was not seen in the Kupffer cells, in contrast to the extensive manner in which that activity was carried on by the reticuloendothelial cells in other organs.

*Lungs.*—The lungs also revealed no significant pathologic change that might be referred to ionizing radiation other than necrosis of the lymphoid elements noted above. The lungs of both control and experimental mice were routinely observed to be quite congested, perhaps incident to the method of killing used, that of asphyxiation with carbon dioxide. This might conceivably mask minor changes. However, our negative findings are in agreement with those of Bloom,[2] who observed no consistent changes in the lungs of animals exposed to external irradiation.

*Blood Vessels.*—An attempt was made to evaluate the state of blood vessels and capillaries in each of the organs studied. Except where specifically stated, no lesions of these structures were seen.

## COMMENT

The foregoing observations of the early pathologic sequelae of the exposure of a large number of mice to an absolute lethal dose of x-rays are summarized in the Table. They are largely in accord with those of many other investigators of radiation illness. However, certain considerations merit special emphasis from the point of view of description and recognition of radiation illness as a pathologic entity and with regard to subsequent efforts to reduce the mortality and morbidity attendant on exposure to ionizing radiation. Chief among these are (*a*) the relative uniformity of the response of lymphoid tissue regardless of its location, (*b*) the morphologic and functional evidence of radioresistance of the reticuloendothelial cells, (*c*) the definite increase in erythrocytophagia, (*d*) the remarkable regenerative powers of the crypt epithelium of the intestine, and (*e*) the radiosensitivity of the spermatogonia of the testis in contrast to the greater radioresistance of not only the more mature forms but also the more primitive prespermatogonia or basement epithelium.

While moderate variations in degree of response could be noted between the spleen and the lymph nodes or the intestinal lymphoid aggregations of any one animal, and even from one lymphoid nodule to another in the spleen, these changes tended to cancel out each other in the entire series, and we conclude that none of these tissues exceeds the other in radiosensitivity. Several factors may be responsible for apparent differences in the degree of their response to irradiation. The studies of Conway[12] which demonstrated the relationship between the seemingly unrelated "germinal centers" and "reaction centers" of lymphoid tissue furnish a clue to this question inasmuch as lymphoid nodules in a state of paucity of lymphocytes, i. e., corresponding to the reaction center of Hellman, may be expected to show a smaller increase in debris following irradiation than corresponding nodules rich in lymphocytes. Local inflammatory states further confuse the picture not only by altering the state of activity of the lymphoid nodule but also by directly changing or obscuring the structure. This is particularly apt to occur in the lymphoid structures draining the intestine and may serve to create an apparent difference in the radiosensitivity of these aggregations and the spleen, for example.

The rarity of morphologic evidence of damage of cells of the reticuloendothelial system following total-body irradiation has been stressed by several observers.[13]

12. Conway, E. A.: Anat. Rec. **69**:487, 1937.

13. Tullis, J. L.: Radioresistant Cells in Certain Radiosensitive Tissues of Swine Exposed to Atomic Bomb Radiation, Arch. Path. **48**:171 (Aug.) 1949.

It is noteworthy that phagocytosis was observed as soon as cell debris began to accumulate and continued until the products of necrosis were removed. Furthermore, since regeneration of hematopoietic elements may come from cells of the reticuloendothelial system in lymphoid centers, bone marrow, and spleen, this system may constitute a significant reservoir of radioresistant cells.

Following total-body exposure to ionizing radiation, an anemia appears which is slowly progressive and has been held by many observers to be due in large measure to disruption of the mechanism by which new erythrocytes are supplied to the blood stream, rather than to an increased rate of attrition of erythrocytes. This is, of course, not solely the case in such animals as swine [14] and man,[15] in which a hemorrhagic diathesis develops and contributes significantly to the anemia. However, our observation of active erythrocytophagia in the spleen and the bone marrow and to a lesser extent in the lymph nodes and the intestinal lymphoid nodules in this series of irradiated mice, with the rather rapid increase in blood pigment in these locations, prompts us to suggest that increased attrition via this mechanism contributes to the anemia of radiation illness. This would be in accord with the conclusions of Schwartz,[16] who has noted a significant increase of bile pigment excretion in dogs, rabbits, and rats, following total body irradiation. Further studies to evaluate possible hemolytic mechanisms involved in radiation illness would appear worth while.

The ability of the intestine to repair its radiation-produced damage with great speed is noteworthy in the light of the severe ulcerations and mucosal sloughs known to occur later in other species subjected to total body irradiation. It has been suggested that the ulcerations arise as a result of focal breakdown of the tissues underlying the mucosa, possibly the lymphoid nodules. That diminished resistance to infection consequent to granulocytopenia also contributes to their production appears probable. It is beyond the scope of this study to evaluate these mechanisms further, but we infer that some mechanism besides the direct effect of the radiation on the intestinal epithelium is responsible for the later pathologic changes seen in the intestine of the totally irradiated animal.

It is noteworthy that whereas spermatogonia were observed to be the most radiosensitive elements of the testis, not only were the older forms more resistant, but so were the prespermatogonia or basement membrane cells. The latter cells appear to give rise to the spermatogonia normally, and probably serve as their source when spermatogenesis returns following irradiation. Their apparent ability to withstand ionizing radiation sufficient to destroy more mature members of the germinal series presents an exception to the law of Bergonie and Tribondeau [17] that primitive cells are more sensitive to ionizing radiation than specialized cells.

## SUMMARY AND CONCLUSIONS

Eight hundred eighty mice exposed to a single absolute lethal dose of total-body x-ray irradiation were killed in groups of 10 at hourly intervals and examined microscopically.

14. Tullis, J. L., and Warren, S.: Gross Autopsy Observations in Animals Exposed at Bikini: Preliminary Report, J. A. M. A. **134**:1155 (Aug. 2) 1947.

15. Warren, S.: Cancer Res. **6**:449, 1946.

16. Schwartz, S.: J. Clin. Invest. to be published; abstracted, Forty-First Annual Meeting of the American Society for Clinical Investigation, Atlantic City, May 2, 1949.

17. Bergonie, J., and Tribondeau, L.: Compt. rend. Acad. sc. **143**:983, 1906.

Pathologic changes were followed in the spleen, lymph nodes and intestinal lymphoid tissue, bone marrow, the small and large intestine, and the testis. No significant changes were observed in the liver or the lungs.

On the basis of these studies we conclude that there is little difference in the degree to which lymphoid tissue is sensitive to a single exposure of total-body irradiation, whether the tissue be in the spleen, the lymph nodes, or the intestinal lymphoid aggregations.

No deleterious effects on the cells of the reticuloendothelial series were observed.

Of the hematopoietic tissues, the erythropoietic series appears to be most sensitive to the effects of ionizing radiation, the granulocytic, or myelopoietic, series next, and the megakaryocytes next. These are all exceeded in sensitivity by the lymphocytes.

The intestinal crypt epithelium of the mouse is quite radiosensitive but has striking regenerative powers. The radiosensitivity of the epithelium of the small intestine is somewhat greater than that of the epithelium of the large intestine.

Of the cells of the testis, the spermatogonia seem to be most radiosensitive and those with dust-like nuclei (the earlier forms) somewhat more sensitive than those with crust-like nuclei. This may be only apparent, and due to the fact that the former naturally mature into the latter. In any case, the still earlier, or more primitive, basement membrane cells seem to be relatively radioresistant.

The Commandant of the Naval Gun Factory, Washington, D. C., and the personnel of the Materials Testing Laboratory of that establishment cooperated in this work. J. S. Otto (HMC) U. S. N., L. H. Dedon (HM3) U. S. N., and D. J. Skelly (HM3) U. S. N., technicians of the Pathology Facility, prepared the specimens. L. B. Coriasso (HM2) U. S. N. was responsible for the handling, hourly killing, and autopsy of the experimental animals.

# A CASE OF ACUTE INFECTIVE ENDOCARDITIS CAUSED BY SALMONELLA DUBLIN

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THIS PAPER describes a case of acute infective endocarditis caused by Salmonella dublin with a fatal outcome after a continued fever of enteric type. Only eight cases of acute endocarditis due to Salmonella organisms have been recorded in the literature. S. dublin has never previously been implicated in this respect, and the present case is therefore thought worthy of reporting.

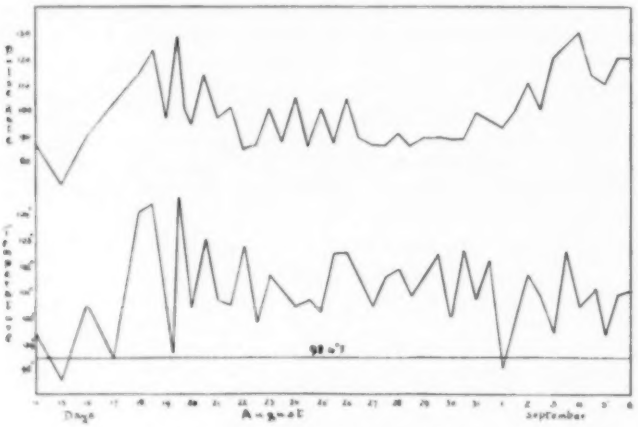

Fig. 1.—Pulse and temperature chart.

## CLINICAL HISTORY

Mrs. H. P., aged 56, on first examination, Aug. 11, 1947, gave a history of two days of shivering attacks with euphoria and slight headache. Her temperature was 99.4 F. and her pulse rate 86 per minute. She had vomited twice and on one occasion had a loose bowel motion. No past history of rheumatic fever or other diseases was obtained.

The patient had an intermittent fever for one week; then she became stuporous, with a temperature of 104 F., a pulse rate of 122, and a respiration rate of 30 per minute. Sulfathiazole, 6 gm. per day, and penicillin, 500,000 units per day, were given. There was no satisfactory response following treatment, and when the patient was admitted to hospital, Aug. 8, her condition had deteriorated. She complained of generalized aches and pains, and her euphoria was even more evident. No alteration in the heart sounds could be detected, possibly owing to the patient's extreme obesity.

From the Department of Pathology, The Royal Masonic Hospital.

On Aug. 28 blood culture yielded a pure growth of S. dublin. The patient's fever became continuous, with only slight remissions, and, despite a dosage of penicillin of 50,000 units every three hours, her condition deteriorated steadily until she died on Sept. 7, after 28 days' illness.

### LABORATORY REPORTS

Aug. 8, 1947. Blood count: hemoglobin, 76% or 10.6 gm./100 cc.; erythrocytes, 3.7 million/c. mm.; color index, 1.0; leucocytes, 5,800/c. mm.; polymorphonuclear granulocytes, 77%; monocytes, 6%; lymphocytes, 17%. Films showed no abnormality of the red blood cells.

Aug. 23. Feces cultures: No organisms of the Salmonella or Shigella groups were isolated. The media used were desoxycholate-citrate agar, Wilson and Blair's medium, tetrathionate broth, and selenite F broth.

Aug. 28. Erythrocyte sedimentation rate (Westergren): 88 mm. in 1 hour; 103 mm. in 2 hours. Blood culture yielded Salmonella organisms later identified as S. dublin.

Sept. 3. Widal reaction: Oxford-standard suspensions were agglutinated by the patient's serum in the following dilutions:

| | | |
|---|---|---|
| S. typhi | H | Less than 1:20 |
| S. typhi | O | 1:2,560 |
| S. typhi | Vi | 1:20 |
| S. paratyphi A | H | Less than 1:20 |
| S. paratyphi A | O | 1:160 |
| S. paratyphi B | H | Less than 1:20 |
| S. paratyphi B | O | 1:640 |
| Nonspecific salmonellas (composite H nonspecific) | | Less than 1:20 |
| Patient's own strain | | 1:1,280 |

Sept. 4. Feces cultures: No organisms of the Salmonella or Shigella groups were isolated.

### AUTOPSY

Autopsy was performed 12 hours after death. External examination showed a very obese woman with a few, small purpuric spots on the right flank and a large bruise above the left elbow.

The heart weighed 422 gm., the hypertrophy being mostly confined to the left ventricle. The aortic valve and the surrounding endocardium were covered with exuberant vegetations (Fig. 2). They were situated chiefly on the aortic surface of the posterior cusps, where they measured 1.0 cm. in diameter and were grayish pink, somewhat firm and rubbery. There were also many smaller vegetations, resembling those, on the ventricular surface of the cusps, which were largely destroyed by the inflammatory process. This had spread to the wall of the right ventricle just above the tricuspid valve, but the endocardium here was intact.

Those parts of the aortic valve which were not affected by the endocarditis looked normal and showed no sign of antecedent inflammation. It was impossible to exclude completely the presence of any preexisting deformity, as the damage to the two posterior cusps was so extensive, the vegetations being continuous over most of the free edges of these cusps. The other heart valves were normal and the coronary arteries patent. The myocardium was rather flabby.

The respiratory system was normal except for pulmonary hypostasis.

In the abdominal cavity, the spleen was enlarged to about twice the normal size, with gray, hyperplastic pulp, but was not diffluent. The liver was pale but of normal size. The gall bladder was filled with bile and appeared normal. The large intestine was distended with gas and contained a small amount of very hard feces. The

contents of the small intestine appeared normal but scanty. There was no enteritis, swelling of Peyer's patches, or enlargement of the mesenteric lymph glands. The kidneys were of normal size, with pale, swollen cortex.

Nothing abnormal was found in the central nervous system.

Fig. 2.—Opened heart showing aortic valve and surrounding endocardium covered with exuberant vegetations.

### HISTOLOGICAL OBSERVATIONS

In sections through the vegetations on the aortic valve, the cusp was buried in large masses of fibrin, penetrated everywhere by small and large colonies of Gram-negative bacilli. Numerous collections of neutrophil leucocytes were also present in many areas. Along the border that includes part of the right auricle, there was a granulomatous reaction with numerous fibrocytes, delicate collagenous fibers and an associated infiltration of large mononuclear cells, plasmacytes, and fewer polymorphonuclear granulocytes and lymphocytes. The myocardium showed focal collections of polymorphonuclear granulocytes in the interstitial tissue.

The kidneys revealed only parenchymatous degeneration; there was no evidence of embolic nephritis.

### BACTERIOLOGICAL STUDIES

A profuse growth of S. dublin in pure culture was obtained from material taken at autopsy from the spleen and the depths of the heart vegetations and from the blood. A few colonies of S. dublin developed in cultures of material from the small intestine, but none in those from the gall bladder.

A heavy growth of a motile Gram-negative bacillus was obtained in blood culture and gave positive slide agglutination with S. typhi O and paratyphi AO and BO sera, as well as with the patient's own serum. The latter finding was helpful, as it suggested immediately that one was dealing with the organism causing the patient's illness. No agglutinations with S. typhi H and S. enteritidis H sera were obtained at this stage. The patient's serum was investigated for the presence of S. typhi Vi agglutinins, and an indefinite positive result in a dilution of 1 : 20 was recorded. A provisional diagnosis and notification of typhoid was made, but the next morning the organism isolated from the patient's blood produced both acid and gas in sugars. The first specimen of serum for the Vi agglutination reaction was slightly hemolyzed, and therefore the result was discounted. The test was repeated on another specimen of serum, which was negative. It was observed that the patient's own serum gave sharp slide agglutination with an organism recovered from a contemporary outbreak of gastroenteritis in Chard, a small town 15 miles (24 kilometers) away from the patient's home. The implication was that the organism isolated in the Chard outbreak was identical with the one from this patient.

The organism gave biochemical reactions typical of a member of the Salmonella group, with the production of acid and gas in glucose, maltose, mannite, and dulcite. Lactose, sucrose, and salicin were not fermented; nor was indole produced from peptone in seven days. When it was found that no H agglutination could be obtained with the sera at the laboratory of isolation, the organism was sent to the Taunton Public Health Laboratory and thence to the Salmonella Reference Laboratory in London. There Joan Taylor showed that it had the antigenic structure O—IX.XII. H—g.p. and was therefore S. dublin.

### EPIDEMIOLOGICAL STUDIES

The source of the infection in this case was not traced. Nineteen days after the onset of the patient's illness, an outbreak of gastroenteritis occurred in Chard near this patient's home in Taunton. This outbreak was subsequently shown to have been caused by S. dublin. The patient with endocarditis gave no history of a recent visit to Chard. The outbreak there affected about 30 people, and in some of them the infection was severe, but in none was septicemia discovered. In Chard 26 cases were reported on Aug. 30, 1947 and a few on the following days. The milk supplied to the patients in Chard came from a single farm, but S. dublin was never isolated from the specimens of milk tested. Investigations were made of feces from the five milkers employed on this farm, one of whom gave a history of a recent attack of diarrhea. S. dublin was isolated from this man and from two others. As regards their infections being the prime source of secondary infections, it must be remembered that they drank the milk as well as handled it. Milk and feces from all the cows on the farm were examined, but no Salmonella organisms were isolated. There was only one calf, and S. dublin was recovered from its feces. The other calves from the farm were not examined, as they had been sold recently and were scattered around the district.

A second isolated case of S. dublin infection occurred in Taunton in the middle of October, 1947. This patient had diarrhea and vomiting. No connection was found between the supplies of milk of this patient and the patient with endocarditis. A second outbreak due to S. dublin, involving both cows and humans, was reported from Long Ashton, North Somerset, in late September, 1947. S. dublin was isolated from two patients with gastroenteritis, although the local physicians stated that at least 50 cases of diarrhea and vomiting had occurred. The distribution of the patients suggested a milk-borne infection, but the organism was not recovered from milk or from persons connected with milking.

In addition there were three outbreaks due to this organism involving cows but not humans in the Glastonbury area of central Somerset in the early autumn of 1947.

## REVIEW OF RECORDED CASES

Before discussing the patient with endocarditis, it is proposed to review other comparable cases in the literature, and for this purpose they will be divided into two groups: (1) cases of Salmonella endocarditis and (2) cases of S. dublin septicemia.

Some important findings in these two groups are summarized in Tables 1 and 2.

*Salmonella Endocarditis.*—This is a rare complication of Salmonella septicemia, as indicated by Wells [1] (1937). In a review of the literature on autopsies of patients infected with S. paratyphi B, he found that endocarditis was observed only once. This appears to have been the case of Kretschmer (Table 1, No. 3), and it is highly questionable whether the endocarditis was caused by S. paratyphi B, as in life this organism was recovered only from the feces, although at autopsy it was grown from the blood, the bile, and the seminal fluid. It is not stated that S. paratyphi B was recovered from the warty vegetations that were on the aortic valves. This man also had boils of the neck, an abscess of the pectoral muscle, and septicemic abscesses of the liver, the kidneys, and the myocardium. The cervical and pectoral abscesses failed to yield S. paratyphi B and the other pyemic abscesses were not cultured. It seems likely, therefore, that these were staphylococcal abscesses and it is more than doubtful that the endocarditis was due to S. paratyphi B.

Another case, which must be regarded as not proved, is that of Gouley and Israel [2] (1934). The patient was a 4-year-old girl with septicemia due to S. choleraesuis. On the ninth day of the illness a double aortic murmur was heard, which later disappeared, and the patient recovered. It was presumed that she had had endocarditis caused by S. choleraesuis. As evidence against this presumption it must be stated that all the other 11 patients died and that in the days before antibiotics it was hardly a condition from which one would expect recovery, especially in a young child.

1. Wells, H. G.: Acute Endocarditis Produced by Bacillus Paratyphosus B, Arch. Path. **23**:270 (Feb.) 1937.

2. Gouley, D. A., and Israel, S. L.: Salmonella Suipestifer Bacteremia with Acute Endocarditis, Arch. Int. Med. **53**:699 (May) 1934.

TABLE 1.—*Cases of Endocarditis Caused by Salmonella Organisms* *

| No. | Authors | Year | Sex | Age, Yr. | Organism | Site of Main Lesion | Previous Heart Disease | Time Ill. Days | Red Blood Cells, Millions | White Blood Cells, Thousands | Polymorphonuclear Granulocytes, % |
|---|---|---|---|---|---|---|---|---|---|---|---|
| 1 | Tenbroeck, Li, and Yu [3] | 1931 | M | .. | S. choleraesuis var. kunzendorf | Mitral valve | ..... | .. | ... | ..... | .. |
| 2 | Gouley and Israel [2] | 1934 | F | 4 | S. choleraesuis | Aortic valve | ..... | 17 | ... | ..... | .. |
| 3 | Kretschmer, W.: Deutsches Arch. klin. Med. **178**: 198. | 1935 | M | .. | S. paratyphi B | Aortic valve | ..... | .. | ... | ..... | .. |
| 4 | Wells [1] | 1937 | M | 50 | S. typhimurium | Mitral valve | Rheumatic | .. | 3.5 | 9.9 | 82 |
| 5 | Meyer, J., and Howell, K. M.: Arch. Path. **26**: 308 (July) | 1938 | M | 36 | S. paratyphi B | Aortic and mitral valves | Rheumatic (aortic and mitral valves) | 18 | ... | 5.5-31.3 | 65-85 |
| 6 | Forster, D. E.: Am. J. M. Sc. **197**: 234..... | 1939 | M | 47 | S. choleraesuis | R. atrium L. ventricle | Syphilitic (aortic valve) | 16 | 5.0 | 11.4-33.4 | 85 |
| 7 | Forster, D. E.: Am. J. M. Sc. **197**: 234..... | 1939 | M | 31 | S. choleraesuis | Mitral valve | Rheumatic | 77 | 5.5 | 16-26 | 78 |
| 8 | Read, C. T.: J. Infect. Dis. **65**: 263..... | 1939 | F | 49 | S. choleraesuis var. kunzendorf | Aortic valve | None | 49 | 5.5-3.9 | 11.7-20 | .. |
| 9 | Goulder and others [4]..... | 1942 | F | 54 | S. choleraesuis var. kunzendorf | Mitral valve | Rheumatic | 21 | 4.5-2.7 | 13.7-21 | 90 |
| 10 | Parker ..... ..... | 1944 | M | .. | S. enteritidis var. chaco | Interventricular abscess and vegetations in both ventricles | ..... | .. | ... | ..... | .. |
| 11 | Anderson, E. S.; Anderson, H. J., and Taylor, J.: J. Path. & Bact. **30**: 533 | 1917 | M | .. | S. fayed | Aortic valve | Congenital deformity of aortic valve | 84 | ... | 6.0 | Normal |
| 12 | Thomas, A. M.: Arch. Path., this issue, p. 408 | 1950 | F | 56 | S. dublin | Aortic valve | None | 28 | 3.7 | 5.8 | 77 |

* A blank space in the table indicates that no information is available on this point.

TABLE 2.—*Cases of Continued Fever Caused by Salmonella Dublin*

| No. | Authors | Year | Sex | Age, Yr. | Recovered or Died | Days of Fever | History of Diarrhea or Vomiting * | Localizing Signs Due to S. Dublin | Source of Organism † | Feces Cultures | Comment |
|---|---|---|---|---|---|---|---|---|---|---|---|
| 1 | Gregg, R. G. S., and Hayes, P.: J. Roy. Army M. Corps **37**: 64 | 1921 | M | Adult | R | 21 | D+++ 24 hr. | None apart from enteritis | Urine 2+ | 1— | Admitted with wound of chest; diarrhea on 10th day, followed by 17 days of fever ‡ |
| 2 | Pesch, K. L.: Zentralbl. Bakt. **98**: 22 (Pt. 1) | 1926 | M | 2/52 | D | 10 | D— V— (? intestinal upset) | Meningitis; otitis media | C.S.F. 2+; blood 2+; bile 2+; urine 1+ | 2+ | P.M. intestines normal ‡ |
| 3 | Bruce White (Bigger) | 1930 | .. | .... | D | .. | ..... | ..... | ..... | .... | Nephrectomy followed by pyrexia |
| 4 | Smith and Scott [7] | 1930 | M | 38 | R | 10+ | D+ at onset | Transient consolidation of lung; hemorrhages of soft palate; rose spots | Blood 2+; urine 5— | 5— | ..... |
| 5 | Smith and Scott [7] | 1930 | F | 2 | R | 42 | D— | Vesicular rash; ulceration of mouth | Blood 1+ | N. r. | Abscess of buttock due to Staph. aureus |
| 6 | Smith and Scott [7] | 1930 | F | Child | R | 6+ | N. r. § | None (?influenza) | Blood 1+ | N. r. | ..... |
| 7 | Smith, J.: J. Hyg. **33**: 224 | 1933 | M | 5 | D | 49 | N. r. | None | Blood 1+ | 1+ | Mastoiditis due to Staph. and hemolytic streptococci |
| 8 | Smith, J.: J. Hyg. **33**: 224 | 1933 | F | 11/52 | R | 3 | V+++; green stools | ?enteritis | Blood 2—; urine 2+, 1— | 3— | ..... |
| 9 | Smith, J.: J. Hyg. **33**: 224 | 1933 | F | 14/52 | D | 6 | N. r. | Meningitis, cough, dullness of chest | C.S.F. 1+; blood not examined | Not examined | ..... |
| 10 | Bridges, R. F.: J. Roy. Army M. Corps **62**: 395 | 1934 | M | 18 | R | .. | N. r. | None | Blood 1+, 1— | N. r. | ..... |
| 11 | Smith, J.: J. Hyg. **34**: 351 | 1934 | M | 7/12 | D | 36 | D— V— | Meningitis; bronchopneumonia | C.S.F. 6+, 3— | N. r. | P. M. intestines normal |
| 12 | Smith, J.: J. Hyg. **34**: 351 | 1934 | F | 2 | D | 3 | N. r. | Tonsillitis; cervical adenitis; bronchopneumonia; empyema | Blood 2+; empyema pus 1+; throat swab 1+; also hemolyt. strep. | N. r. | P. M. intestines normal |
| 13 | Davidson, L. S. P., and Fullerton, A. W.: Quart. J. Med. **8**: 43 | 1938 | F | 25 | R | .. | V+ and collapse ?due to hemolysis | Hemolytic crisis in a latent case of hemolytic anemia precipitated by infection | Blood 1+ | Frequently + | ..... |
| 14 | Rohm | 1938 | .. | 10/12 | R | .. | D— V— | Osteomyelitis of metatarsal | Pus from bone | N. r. | Recovered; no general or intestinal symptoms |
| 15 | Rohm | 1938 | M | 6/12 | D | 6 | D— V 2+ | Meningitis | C8F. S. Gaertner and pneumococci 1+; blood 1+; urine 1+ | 1+ | P. M. intestines normal |
| 16 | Guthrie, K. J., and Montgomery, G. L.: J. Path. & Bact. **49**: 393 | 1939 | M | 9/12 | D | 21 | V+; green stools | Meningitis and vesicular rash | C.S.F. 1+ | N. r. in vivo | P. M. S. dublin from meninges, gallbladder, spleen, Peyer's patches, cecum, and mesenteric gland; catarrhal enteritis; cholangitis |
| 17 | Thomas, A. M.: Arch. Path., this issue, p. 408 | 1949 | F | 56 | D | 28 | V twice; loose motions 24 hr. | Endocarditis | Blood 1+ | 2— in vivo | P. M. S. dublin from heart, spleen, small intestine; intestines normal; gallbladder normal |

* D+++ indicates severe diarrhea; D—, no diarrhea; V+, slight vomiting; V—, no vomiting.
† Urine 2+ indicates 2 positive urine cultures; blood 1—, 1 negative blood culture.
‡ The organisms from cases 1 and 2 were initially identified as S. enteritidis Gaertner but were subsequently shown by Smith and Scott (1930) to be S. dublin.
§ N. r. indicates not recorded.

The other pitfalls in the literature are in connection with nomenclature. The organism isolated by Tenbroeck, Li, and Yü [3] (1931) is reported as being S. paratyphi C, but it did not ferment arabinose and is stated by them to be identical with nonspecific hog cholera bacilli. In so far as it has been conventional to distinguish between these two organisms, that of Tenbroeck, Li, and Yü is here recorded as S. choleraesuis var. kunzendorf and not as S. paratyphi C. The organism of Well's case is referred to as S. paratyphi B, but was ultimately identified as S. typhimurium. When the above sources of error are taken into account, one finds then records of eight authentic cases of Salmonella endocarditis in the literature.

In addition to these, Parker, in a personal communication, stated that in Bengal during 1943 and 1944 he saw about 30 cases of septicemia in Indian troops caused by S. enteritidis var. chaco. One of these patients, at first considered to be mildly ill, by the third week had a hectic temperature, his condition deteriorating and death occurring in the fifth week. An abscess of the interventricular septum, with large vegetations protruding into each ventricle, was the only significant autopsy finding.

*Salmonella Dublin Septicemias.*—S. dublin was first differentiated from S. enteritidis by Bruce White [4] (1930). The type strain was isolated from a fatal case of continued fever following a kidney operation. Savage [5] (1940), in reviewing Salmonella infections in various hosts, noted that calves and cattle are the main reservoirs of S. dublin. Recent information as to its occurrence in cattle is contained in an excellent paper by Field [6] (1949). Although he isolated S. dublin from cattle on 66 farms during the survey, no outbreak of food poisoning traceable to cattle occurred in the area under examination. In cattle S. dublin may cause a general systemic infection, but in calves, particularly, it produces diarrhea. In man, S. dublin may produce (*a*) no symptoms at all, (*b*) classic acute gastroenteritis, (*c*) septicemia without localizing signs, or (*d*) septicemia with local septic manifestations, notably meningitis.

Cases belonging to the last two groups are summarized in Table 2. Difficulty with the nomenclature of the organism has again arisen here, as those from the first two cases in the table were initially identified as S. enteritidis Gaertner. They were later reexamined by Smith and Scott [7] (1930) and shown to be S. dublin.

## COMMENT

From the study of the present case and a survey of the relevant cases in the literature (Table 2) it is possible to draw certain conclusions:

### Salmonella Endocarditis Cases

1. Seven different Salmonellas have been implicated as a cause of acute endocarditis in 12 cases. S. choleraesuis and its kunzendorf variant were involved in four and three cases, respectively.

3. Tenbroeck, C.: Li, C. P., and Yü, H.: J. Exper. Med. **53**:307, 1931.
4. White, P. B.: J. Hyg. **24**:443, 1930.
5. Savage, W., and others: Proc. Roy. Soc. Med. **33**:357, 1940.
6. Field, H. I.: Vet. J. **104**:8, 1948.
7. Smith, J., and Scott, W. M.: J. Hyg. **30**:32, 1930.

2. Antecedent damage to the heart valves was established in six cases; in four of these it was rheumatic mitral disease, in one syphilitic involvement, and in one a congenital deformity of the aortic valve. No evidence of previous damage or history of heart disease was obtained in two cases, in which the aortic valves showed the main lesion.

3. Hematological findings include a mild to moderate degree of anemia with polymorphonuclear granulocytosis. Also a raised erythrocyte sedimentation rate was present in cases 9 and 12, the readings being, respectively, 44 and 88 mm. in one hour (Westergren method).

4. Tachycardia of some degree was noted in five cases, Nos. 5, 6, 7, 11, and 12 showing pulse rates of 100 per minute or more. Apart from agonal readings, the highest figures were obtained in the present case, but in most of the cases recorded tachycardia was not a marked feature. This may well be due to the fact that sometimes only one pulse rate was noted, that being the one taken on admission to hospital.

5. Death occurred in 11 of the 12 cases. The case in which the patient survived, that of Gouley and Israel,[2] cannot be regarded as confirmed.

### Salmonella Dublin Cases

*Age.*—Five adults and 10 children are included. Fatal meningitis occurred in four children under 1 year old. The case of endocarditis is the only instance of a localized lesion produced by S. dublin in an adult.

*Mortality.*—Eight of 15 patients died. They included six children and two adults. Except for the patient in the present case, the only infected adult who died was the patient whose illness was associated with nephrectomy, described by Bigger (Table 2, No. 3), about which no information is available.

*Local Manifestations.*—S. dublin was isolated from, and accepted as the cause of, the local lesion in four cases of meningitis, one of pneumonia, and one of endocarditis. All of the patients died. Less important localizing signs, from which no attempt was made to isolate S. dublin, included vesicular rashes in two patients and rose spots in one. Four patients had indefinite chest symptoms as a secondary feature. In some patients local manifestations occurred which were clearly not due to S. dublin but to some accompanying infection; Cases 5 and 7 are examples of this.

*Septicemia.*—Septicemia without conspicuous and effective localization occurred in seven cases, Nos. 1, 2, 4, 5, 6, 8, and 10. All the patients recovered.

*Duration.*—The patients seem to fall into two groups, those with the fulminating type of infection, lasting three to six days, and those with the more prolonged type, lasting from three to six weeks. Two young infants with signs of meningitis lived for three or more weeks.

*Source of the Organism.*—S. dublin was isolated from blood alone in six cases, from cerebrospinal fluid alone in three cases, from urine alone in two cases, from blood and feces in two cases, and from multiple sources in two cases.

*Gastroenteritis.*—This was usually conspicuous by its absence. A definite history of brisk diarrhea at the onset of the disease was obtained in one patient only. A complete absence of diarrhea was noted in two cases, and none was recorded in five. Sometimes there was a vague history of the patient having vomited once or

having had an intestinal upset. In none of the cases in which autopsies were performed was there any evidence of enteritis. Also, from antemortem feces S. dublin was isolated in three cases but was not isolated in four cases and not reported in eight. The culture media employed are not recorded.

*Associated Conditions.*—The debilitating illnesses associated with S. dublin septicemias are particularly interesting; they occurred in Cases 1, 3, 5, and 7 of Table 2. The patients in the first, third, and fifth cases seem to have acquired their S. dublin infection while in hospital for some other condition. The patient in Case 7 appears to have had his resistance so lowered by the S. dublin infection that a coccal mastoiditis developed, to which he ultimately succumbed. Goulder and associates[8] (1942) noted the connection between debilitating diseases and septicemic infections caused by S. choleraesuis; this association also seems to exist with S. dublin.

*Epidemiological Aspects.*—The source of the infection was not traced in the sporadic or septicemic cases, whereas in all outbreaks of gastroenteritis due to S. dublin in Somerset there were no cases of septicemia. Apart from the resistance of the host, two variables may possibly be concerned in the distinction between the septicemic and the gastroenteritic cases. These are the size of the infecting dose and the virulence of the infecting strain. The presumption is that in an epidemic the infecting dose is large, particularly in milk-borne outbreaks, milk being the commonest vehicle for S. dublin. In isolated septicemic cases the number of organisms in the initial infecting stage may well be small and excite a minimum of response. This theory is supported by the vague history of looseness of the bowels and vomiting which may accompany the septicemic cases. It would also seem conversely that when a brisk initial reaction is produced, as in patients with acute gastroenteritis, prolonged septicemia is not likely to be established and that the prognosis is therefore good. Field[6] (1948) suggested that low pathogenicity of S. dublin may be responsible for a lack of human gastroenteritis outbreaks in the presence of a high incidence of carriers and of clinical cases in cattle. The importance of the dosage of Salmonella organisms has been shown by Hormaeche and associates[9] (1936), who demonstrated that adults can ingest 2,000 to 4,000 million S. typhimurium with only slight diarrhea and no other symptoms. On the other hand, S. typhi after ingestion may produce a clinical case of typhoid with only a few organisms.

*Comparison of S. Dublin and S. Choleraesuis Septicemia.*—Many of the conclusions with regard to S. dublin septicemia correspond with those reached by Harvey[10] (1937) in connection with septicemia caused by S. choleraesuis. He concluded that the latter organism is of low virulence and causes septicemia most commonly in young children, who have little resistance. His patients also showed slight anemia, and, with localization, leucocytosis and increase of mortality. There is also an association with debilitating conditions and surgical procedures.

8. Goulder, N. E., and others: New England J. Med. **226**:127, 1942.

9. Hormaeche, E.; Peluffo, C. A., and Aleppo, P. L.: Arch. urug. med. **9**:113, 1936.

10. Harvey, A. M.: Salmonella Suipestifer Infection in Human Beings: Review of Literature and Report of 21 New Cases, Arch. Int. Med. **59**:118 (Jan.) 1937.

The terms "septicemia" and "prolonged fever" used in connection with S. dublin infections have been interpreted broadly, the intention being to include all S. dublin infections which are not instances of classic, acute gastroenteritis.

## SUMMARY

A case of acute endocarditis caused by S. dublin is described, with the clinical pathological, and autopsy findings.

The previously recorded cases of Salmonella endocarditis are reviewed and summarized in Table 1.

Cases of S. dublin septicemia described in the literature are reviewed and summarized in Table 2.

A number of conclusions are drawn from the present case and from those already reported.

Dr. G. Lyon-Smith gave me permission to publish this case and furnished the clinical details. Dr. J. Boycott, of the Taunton Public Health Laboratory, helped with the bacteriological investigation of the organism and supplied notes of the local outbreaks of gastroenteritis. Dr. M. T. Parker contributed information about his cases in India, as well as precise and helpful criticism.

# EXPERIMENTAL ATHEROARTERIOSCLEROSIS

## Localization of Lipids in Experimental Arterial Lesions of Rabbits with Hypercholesteremia

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THERE are three common forms of human arteriosclerosis. One is characterized principally by degenerative changes in the media; the second, by proliferation of the intima, and the third, by atheromatous changes in the proliferated intima and degenerate media. The three types of disease frequently occur together in the same vascular segment. This has raised the question whether there is a basic cause, common to all, or whether the etiologic factors may differ, with one form of the disease facilitating the development of another.

Previous experiments showed that acute medial degeneration produced by local freezing of the aortic wall was followed by local intimal proliferation, which was more active in young than in old rabbits.[1] The proliferating intima provided a framework within which a new vascular wall composed of well-oriented collagen, elastic tissue, and smooth muscle was formed. This intimal reaction to medial degeneration often resembled the fibroelastic form of human intimal arteriosclerosis.

The present study was designed to determine the effect of hypercholesteremia on the sequences of local medial degeneration and intimal regeneration at sites of aortic and arterial lesions produced by freezing in young and old rabbits.

### METHODS

Twenty-three male rabbits of the following species were used: New Zealand white, 17; brown Belgian hare, 2; German checker, 2; black and white, 1; gray chinchilla, 1. Eleven animals were more than 100 weeks old and 12 were about 20 weeks old at the beginning of the experiment. The duration of the experiments varied from 25 to 76 weeks with old animals and from 27 to 73 weeks with young animals. The average duration of the experiments was 39 weeks with old animals and 46 weeks with young animals. The average age of old animals during the experiment was 156 weeks and that of young animals 43 weeks.

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This study was supported by funds from the National Heart Institute and the Otho S. A. Sprague Memorial Institute.

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1. (*a*) Taylor, C. B.; Baldwin, D., and Hass, G. M.: Localized Arteriosclerotic Lesions Induced in the Aorta of the Juvenile Rabbit by Freezing, Arch. Path. **49**:623 (June) 1950. (*b*) Baldwin, D.; Taylor, C. B., and Hass, G. M.: A Comparison of Arteriosclerotic Lesions Produced in Young and in Old Rabbits by Freezing the Aorta, ibid. **50**:122 (July) 1950.

The beginning of the experiment was on the date when animals were placed on a diet rich in cholesterol. The diet was prepared as follows. Total lipid extracts of sheep spinal cords were prepared and the cholesterol removed.[2] The extracts had the following average composition: cerebrosides, 36.5%; lecithin, 25.3%; cephalin, 14.0%; alcohol-insoluble waxes, 20.7%; cholesterol, 0.1%; residue, 3.4%. Cholesterol (chemically pure) was added to the extract so that its final concentration was 15.0%. Proper amounts of the cerebral lipid-cholesterol mixture were dissolved in petroleum benzin and mixed with purina® pellets. The petroleum benzin was allowed to evaporate. The final prepared diets contained 1.0, 1.5, 2.0, 2.5, and 3.0% of the cerebral lipid-cholesterol mixture.

The diet of the animals was varied according to their weight and the rise in their total blood cholesterol values. In general, a diet consisting of 13.3 gm. of purina® pellets impregnated with 1.5 to 3.0% of the lipid-cholesterol mixture for each pound of body weight was satisfactory. With this diet the maximum daily intake of cholesterol was 40 to 80 mg. per pound of body weight.

Animals were weighed initially, and a record of changes in weight was kept.

Total blood cholesterol values were determined in duplicate by the Sperry-Schoenheimer method.[3] Analyses were made before institution of the diet and at monthly intervals thereafter. Average values were calculated from the data for the period before and the period after the production of vascular lesions by freezing.

Vascular lesions were made by local freezing of the vessel in a manner previously described.[1a] Lesions were not produced until animals had been on the diet for 22 weeks. This interval was chosen as suitable because previous experience had shown that the first significant traces of aortic atherosclerosis usually did not become grossly apparent until animals had been fed the diet for a period of about 22 weeks. It was desirable that degenerative and regenerative changes should occur in the lesions during the initial period of active atheromatous deposition.

Five vascular lesions were produced in each animal. Three were in the wall of the aorta. One was just distal to the origin of the renal arteries. The second was located midway between the origin of the renal and that of the common iliac arteries. The third was just proximal to the bifurcation of the aorta. The two remaining lesions were produced in the wall of the left renal artery and the wall of the left common iliac artery at distances of 10 to 20 mm. beyond the points of origin of these arteries.

Animals were killed at intervals varying from 2 to 52 weeks after the production of vascular lesions. The average age of the lesions was 17 weeks in the old animals and 24 weeks in the young ones. At autopsy, tissues were taken from all organs, fixed in formalin, and prepared in sections for routine microscopic study. The aorta and its major branches were removed *en bloc*. A plot was made of visible atheromatous lesions above the level of the origin of the renal arteries. The per cent of the intima involved by atheromatous plaques was calculated from the plot. Representative segments of the arch and of the thoracic portions of the aorta were taken for control microscopic study. The three lesions produced by freezing the lower portion of the abdominal aorta were resected transversely, care being taken to include adequate amounts of aortic wall above and below each lesion. These blocks were fixed in formalin, embedded in paraffin, and cut transversely in serial sections so that microscopic changes above and below each lesion could be compared with those at the site of the lesion. Lesions produced by freezing the renal artery and the common iliac artery, together with control segments of the opposite, unfrozen arteries, were similarly prepared for microscopic study. All serial sections were stained with hematoxylin and eosin. Representative sections were thereafter decolorized and stained by Weigert's method for demonstrating elastic tissue. The combined studies gave us an opportunity to survey the vascular system in general and to make comparisons between atheromatous disease at the sites of lesions and the disease in the contiguous, as well as in the control paired, vascular segments.

2. Armour & Company supplied these extracts.

3. Sperry, W. M., and Webb, M.: A Revision of the Schoenheimer-Sperry Method for Cholesterol Determination, J. Biol. Chem. **187**:97, 1950.

## RESULTS

Young animals tolerated the long experiment better than old animals. The range of weight of young animals was 4.0 to 5.8 lb. (1.8 to 2.6 kg.) at the beginning of the experiment and 5.25 to 8 lb. (2.4 to 3.6 kg.) at the end. The range of weight of old animals was 8 to 10.5 lb. (3.6 to 4.7 kg.) at the beginning and 5 to 8.7 lb. (2.2 to 3.9 kg.) at the end. As a rule, young animals gained weight more slowly than normal, while old animals slowly lost weight for weeks and then suddenly lost weight for two to three weeks before death. Ulcerations of hindpaws occurred after prolonged periods of the diet. They were apparently preceded by accumulation of lipids in macrophages in the corium of the skin of the paws. Presumably, the infected ulcers contributed to the debility of many animals but were not responsible for the retardation of growth and well-being of all animals.

The blood cholesterol values were determined initially. The range in young animals was 26 to 107 mg., with an average of 69 mg., per 100 cc. The range in old animals was 25 to 96 mg., with an average of 52 mg., per 100 cc. After institution of the diet, the total blood cholesterol increased at a fairly rapid rate in most animals. The average blood cholesterol level during the first 22 weeks was 375 to 1,700 mg. per 100 cc. in old animals and 140 to 870 mg. per 100 cc. in young animals. The average blood cholesterol level of old animals was 751 mg. per 100 cc. and that of young animals was 486 mg. per 100 cc. After 22 weeks of the diet, arterial lesions were produced by freezing. Thereafter, the blood cholesterol usually continued to rise so that the postoperative average values ranged from 650 to 2,450 mg. per 100 cc. in old animals and from 360 to 1,600 mg. per 100 cc. in young animals. The over-all average in the postoperative period was 1,219 mg. per 100 cc. for old animals and 982 mg. per 100 cc. for young animals. These conditions were suitable for the development of hypercholesteremic aortic atherosclerosis within the period of duration of all experiments.

## GROSS EXAMINATION OF TISSUES

At autopsy, gross examination of organs of animals disclosed changes similar to those which have been observed in hypercholesteremic rabbits by other authors.[4] The aorta showed the customary distribution of atheromatous intimal lesions. These were most conspicuous in the arch of the aorta and diminished distally in size and number. In the thoracic and abdominal aorta the elective site of localization of lesions was around the orifices of branches. Small branches rarely contained atheromas, but larger branches usually showed a few small plaques, which diminished in number and size with increasing distance from the point of origin of the vessel. As a rule, the abdominal aorta below the level of the renal arteries had only a few small intimal atheromas. This was especially true when less than 80% of the intima of the aorta proximal to the origin of the renal arteries was involved by atheromatous deposits. The extent of involvement of this part of the aorta varied from 6 to 99%, with an average of 21% for the older group and 51% for the young

4. Hueper, W. C.: Arteriosclerosis, Arch. Path. **38**:162 (Sept.), 245 (Oct.), 350 (Nov.) 1944; **39**:51 (Jan.), 117 (Feb.), 187 (March) 1945.

animals. These data supported the impression that young animals were more likely than old animals to have severe atheromatosis under similar conditions. The reasons for this were not apparent. The best correlation that was made was as follows: The logarithm of the per cent of aortic intimal involvement was directly proportional to the logarithm of the product of the average of total blood cholesterol values in excess of 200 mg. per 100 cc. and the duration of feeding and was inversely proportional to the logarithm of the average age of the animal during this period. The proportionality factor varied from 0.8922 to 2.1686 with different animals, so that this formulation of factors governing atherosclerosis left much to be desired.

The aorta and arteries in the region of lesions produced by freezing showed slight induration and thickening of their walls. Occasionally, the thickened areas were yellowish white. Inasmuch as transverse serial sections were desired for microscopic study, the vessels were not opened for internal inspection.

### GENERAL MICROSCOPIC STUDY OF TISSUES

Routine microscopic study of organs and tissues other than those involved in the lesions produced by freezing disclosed systemic changes generally encountered in hypercholesteremic rabbits.[4] We were impressed by the elective localization of atheromatous disease in certain extra-aortic vascular systems. The coronary arterial system was the most seriously affected part of the vascular system. The degree of involvement of coronary arteries could not be positively correlated with the degree of involvement of the aorta. In most cases a better prediction of the magnitude and the duration of the elevated blood cholesterol level could be made from an estimate of the severity of coronary arterial rather than aortic atherosclerosis. Severe coronary arterial disease was usually accompanied by evidence of small myocardial infarcts in various stages of healing.

The pulmonary arterial system showed atheromatous lesions almost as often as the coronary arterial system. There was an inconstant rough correlation between the relative magnitudes of pulmonary and coronary arterial atherosclerosis. However, the amount of pulmonary atherosclerosis was never as great as the amount of coronary atherosclerosis in the same animal. Furthermore, pulmonary arterial disease was primarily in large vessels, while small intramyocardial branches of the coronary arterial system were usually severely involved.

The branches of the celiac axis and the superior mesenteric artery were occasionally slightly atheromatous. Usually the conspicuous lesions were in the intramuscular course of pyloric and duodenal arteries.

The remainder of the peripheral arterial system was largely free from significant atheromatous changes. This was especially true of small arteries and arterioles within the parenchyma of various organs.

Microscopic study of the principal organs showed the changes usually encountered in hypercholesteremic rabbits.[4] In addition, several animals had amyloid disease, presumably secondary to infected ulcerations of hindpaws. The amyloid deposits were conspicuous in the spleen, the pancreas, and the kidneys. At times, the kidneys showed typical glomerulotubular changes of amyloid nephrosis. No

correlation could be made between the severity of atherosclerosis, amyloid nephrosis, and hypercholesteremia. Similar changes have recently been described by others.[5]

The microscopic appearance of the atheromatous lesions of the aorta and other vessels varied considerably. At one extreme the lesions were composed of masses of lipid-laden macrophages (Figs. 1 and 2). At the other extreme the lesions consisted of a delicate web-like reticular structure enclosing lipid deposits, which were often in crystalline form (Fig. 3). Many lesions were a composite of these two extremes, the web-like structure and macrophages being present in a variable ratio (Fig. 2). In general, the web-like reticular structure predominated in atheromatous lesions of the aorta and its major branches, especially in small arterial branches of

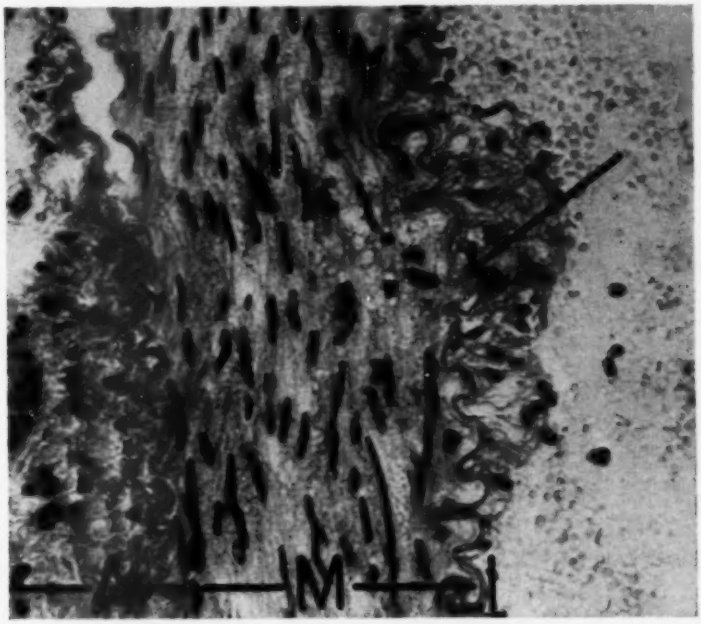


Fig. 1.—Photomicrograph of a very early stage of the development of an atheroma of an iliac artery. The locations of intima (*I*), media (*M*), and adventitia (*A*) are indicated. The intimal accumulation of lipophages overlies a small discontinuity, indicated by the arrow, in the internal elastic membrane. Beneath this discontinuity, in the adjacent media, there is an extracellular accumulation of lipid droplets, as well as a few migrating macrophages. In this early spontaneous lesion of an artery there seems to be a relation between the intimal disease, the discontinuity of the internal elastic membrane, and the medial disease.

the coronary and gastrointestinal arterial systems. On the other hand, the lesions of the pulmonary arteries were usually composed of masses of lipid-laden macrophages.

The media of the large vessels, with rare exceptions, retained a normal structure even in the instances of most severe atheromatosis of the intima. Occasionally,

5. Hoffman, I.; Friedfeld, L.; Fishberg, E. H., and Fishberg, A. M.: Amyloidosis and Hypertension in Cholesterol-Fed Rabbits, Proc. Soc. Exper. Biol. & Med. **78**:37, 1951.

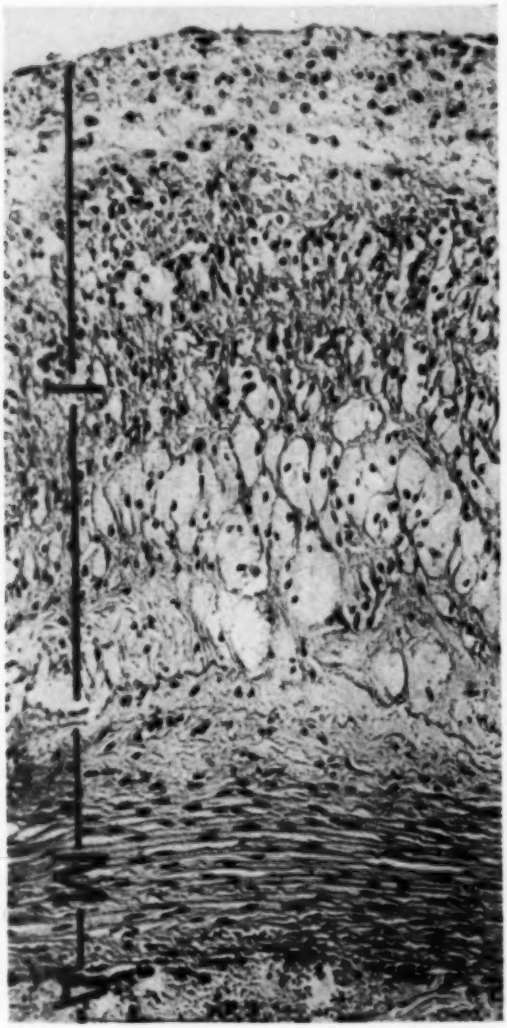

Fig. 2.—Photomicrograph of the most severe form of spontaneous aortic atherosclerosis. The extent of the intimal lesion (*I*), the media (*M*), and the adventitia (*A*) is indicated. There is about as much fibroelastic and smooth-muscle regenerative activity here as is ever encountered in the evolution of spontaneous plaques. This proliferative activity is pronounced over the surface of the lesion. More deeply, there are many macrophages, some of which have degenerated, leaving crystals of lipids in a loosely fibrillar meshwork. These changes have affected the inner one-fourth of the media, leading to interruption of elastic networks and disappearance of the normal structure of the media. This illustrates the maximum extent to which the aortic media is involved by spontaneous atherosclerosis. Furthermore, complete destruction of all cells of the aortic media by freezing does not lead to any greater atheromatosis of the media than is shown here.

lipophages and atheromatous deposits were found between the innermost elastic lamellae of the aorta but the remainder of the media retained an essentially normal structure, despite lipid droplets in smooth muscle cells and interlamellar connective tissue (Fig. 2).

The media of the smaller arteries, however, frequently had lipid deposits in smooth muscle cells, and waxy web-like lipid deposits in the media occasionally were continuous through defective internal elastic membranes with contiguous intimal atheromatous lesions (Fig. 1). The latter change was rarely found anywhere except in the coronary arterial system.

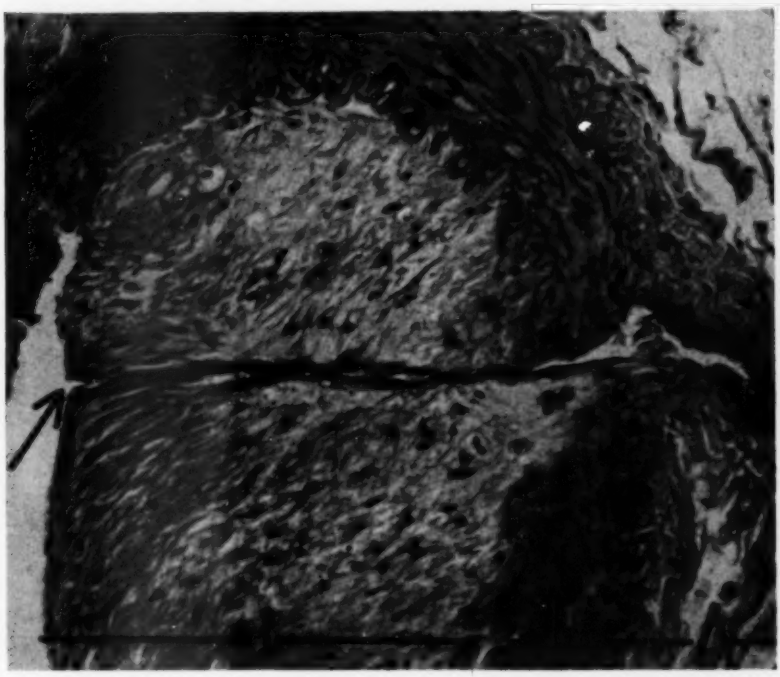

Fig. 3.—Photomicrograph of the usual structure of the web-like reticular plaque, which presumably represents a late stage of the evolution of the lipophage plaque of spontaneous atherosclerosis. This section shows a plaque formed around the orifice of a small branch of the common iliac artery. The arrow points to the orifice of the narrow channel leading from the lumen of the common iliac artery through the reticular plaque into the small arterial branch. The thicknesses of the intimal plaque (*I*), the media (*M*), and the adventitia (*A*) of the common iliac artery are indicated. This form of intimal plaque is more common in the muscular arteries than lipophage plaques, which occur more frequently in the aorta and the pulmonary artery.

Proliferative fibroelastic intimal changes were found in only two of several hundred lesions examined (Fig. 2). In other words, the common form of fibroelastic hyaline intimal change so characteristic of human senile arteriosclerosis did not develop to a significant degree in atheromatous plaques even though degeneration and disintegration of plaques were common.

MICROSCOPIC STUDY OF VASCULAR LESIONS PRODUCED BY FREEZING

Sixty-five lesions of the aorta produced by freezing the vessel at three levels between the origin of the renal arteries and the aortic bifurcation were studied in serial microscopic sections. Of these, 44 showed good evidence of atheromatous localization (Figs. 4 and 5, left). Eight showed evidence which might be interpreted as due to atheromatous localization. Thirteen showed no evidence of localization of atheromatous deposits. Lesions in which no evidence of atheromatous change was encountered were, with one exception, less than nine weeks old. Most lesions in which there was questionable evidence of atheromatous change were less than 16 weeks old. Ordinarily, when atheromatous changes were found in one lesion, similar

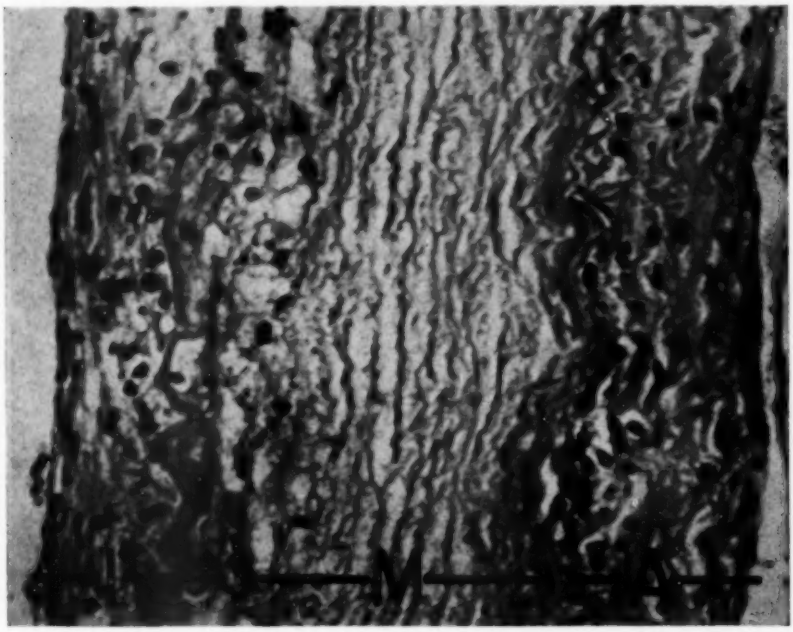

Fig. 4.—Photomicrograph of a lesion, two weeks old, produced by freezing the wall of the lower portion of the abdominal aorta of a rabbit with a blood cholesterol level of 640 mg. per 100 cc. In this animal, 9% of the intima of the thoracic aorta was involved by atheromas, and there were no microscopically demonstrable spontaneous atheromas of the lower portion of the abdominal aorta. The extent of the proliferated intima (*I*), the degenerate media (*M*), and the adventitia (*A*) is indicated. Note the infiltration of lipophages throughout the proliferated intima and the immediately adjacent media. This shows the maximum degree of interruption and distortion of intimal proliferation, as well as the early elective localizing of lipids at the site of injury and repair of the aortic wall. The partial preservation of the elastic networks of the degenerate media shown here is a constant finding at the site of lesions produced by freezing the aorta.

changes were found in the other two aortic lesions. The proximal lesions, however, showed a greater tendency to undergo atheromatous changes. This had no constant relationship to the proximity or the magnitude of atheromatosis of the thoracic and abdominal aorta. Indeed, the intima of the abdominal aorta above and below most

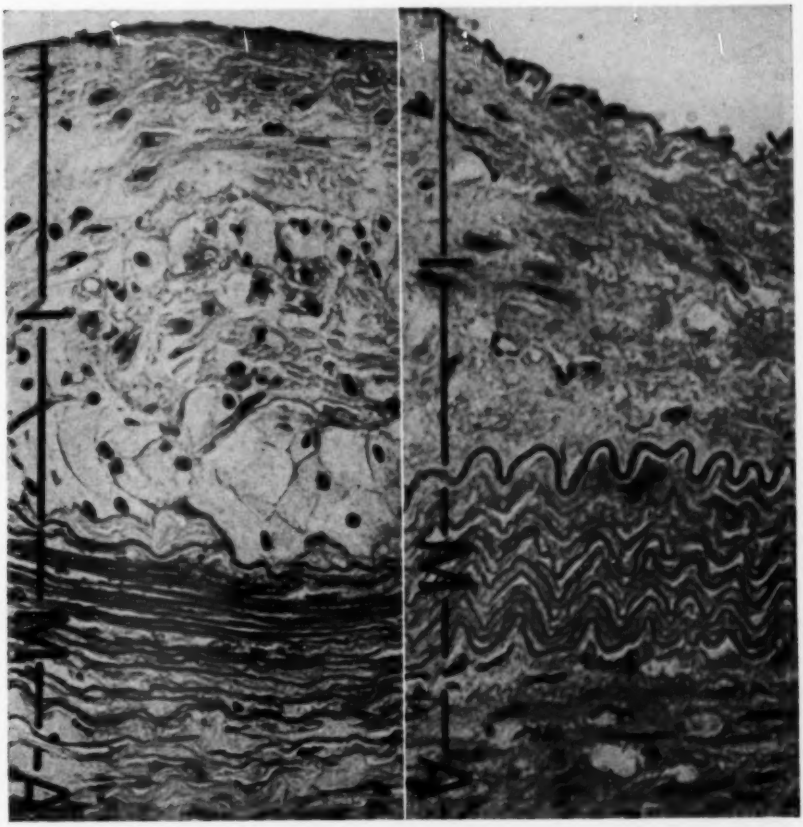


Fig. 5.—Left: Photomicrograph of a lesion, 26 weeks old, produced by freezing the wall of the lower part of the abdominal aorta of a rabbit with an average blood cholesterol value of 1,090 mg. per 100 cc. In this animal, 70% of the intima of the thoracic aorta was involved by atheromas, and there were no microscopically demonstrable spontaneous atheromas of the lower part of the abdominal aorta. The extent of the proliferated intima (*I*), the degenerate media (*M*), and the adventitia (*A*) is indicated. Note that the lipophage infiltration is restricted to the inner one-half of the proliferated intima. This has interrupted the usual regenerative sequences of intimal repair following freezing of the aortic wall. Fibroblasts are less numerous, and the newly formed fibroelastic tissue has a peculiar loose reticular character resembling that found in "mucinous" degeneration of proliferated intima in human arteriosclerosis.

Right: Photomicrograph of a lesion, 52 weeks old, produced by freezing the wall of the lower part of the abdominal aorta of a rabbit with an average blood cholesterol value of 560 mg. per 100 cc. In this animal, 36% of the intima of the thoracic aorta was involved by atheromas, and there were few microscopic spontaneous atheromas of the lower abdominal aorta. The extent of the proliferated intima (*I*), the degenerate media (*M*), and the adventitia (*A*) is indicated. Note the absence of significant lipophage infiltration in the proliferated intima, though the interfibrillar spaces contain scattered lipid droplets. The regenerated intimal fibroelastic tissue is of poor quality. Fibroblasts are few. There are no smooth muscle cells. The fibroelastic tissue is composed of thick, irregular, fused fibrils, having the appearance of fibrils encountered in "hyaline" degeneration. This appearance grades into the more advanced "hyaline" changes shown in Figure 7, right.

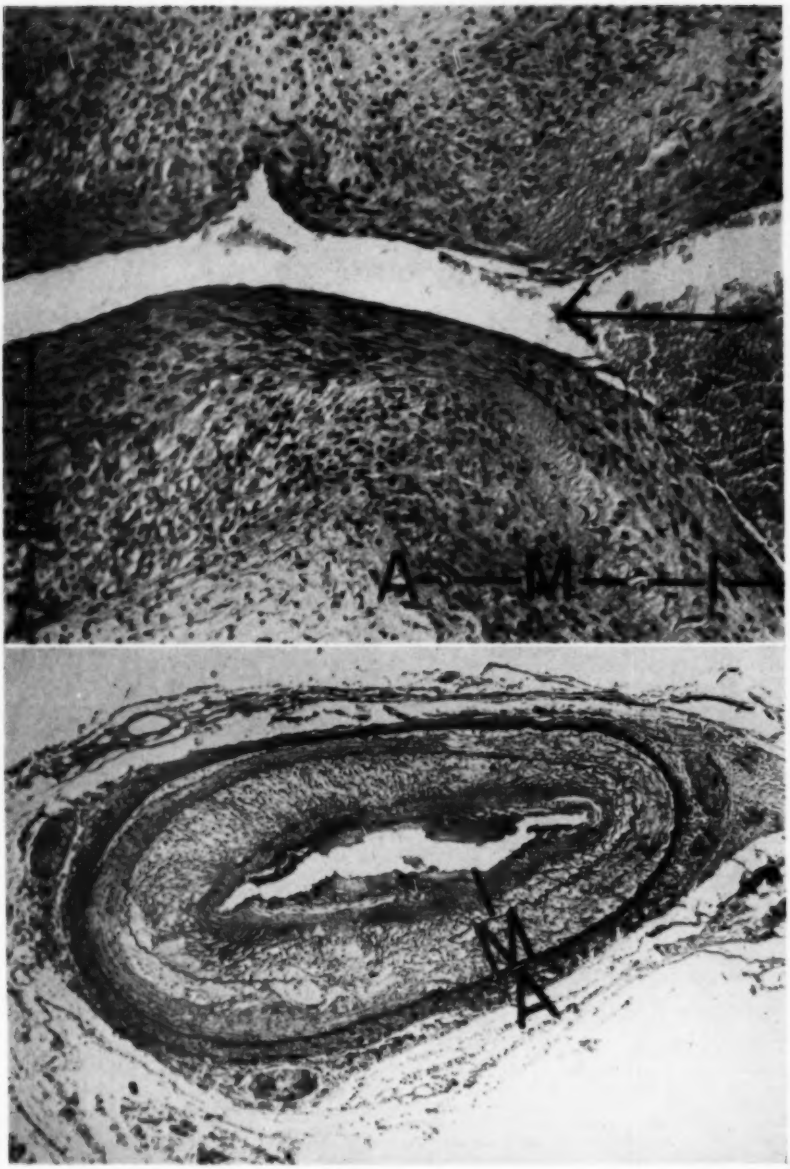


Figure 6

(See legend on opposite page)

lesions was usually free from significant atherosclerotic change. Seldom could any continuity be established in the serial sections. When continuous changes were found, they were appropriately evaluated in interpreting the atherosclerotic changes within the lesions.

The lesions of least age showed the usual sequences described previously as following medial degeneration produced by freezing the aortic wall.[1] Briefly, this consisted of immediate death and rapid dissolution of all smooth muscle cells, with little or no accompanying inflammatory reaction in the media. Within a few hours intimal proliferation began. Later, collagen was deposited between proliferating cells as the intimal plaques thickened. Still later, elastic fibrils and smooth muscle cells appeared in the proliferated intima. Finally, a well-oriented system of cells and fibrils was formed. This system was ordinarily as thick as the original aortic wall and closely resembled the aorta in most essential characteristics. The completion of this regenerative process required about 9 to 12 weeks.

The sequences of medial aortic degeneration and regeneration observed previously in normocholesteremic animals were similar to those found in the present group of hypercholesteremic animals. There was one notable difference. Previous experiments had shown that calcium salts were quickly deposited and slowly resorbed in the degenerate media of normocholesteremic animals.[1] Calcium salts in significant amounts as judged microscopically were rarely encountered in the degenerate media of the present group of hypercholesteremic animals. Calcium deposits were recognizable as amorphous deposits in only two lesions of one animal and as bone in one lesion of another animal. Lipophagic and atheromatous permeation of the breadth of the degenerate aortic media did not occur in lesions of hypercholesteremic rabbits (Figs. 4 and 5).

The sequences and final pattern of regenerating intima previously described in normocholesteremic animals was usually modified in hypercholesteremic rabbits. Regeneration of intima was retarded by atheromatous localization among proliferating intimal cells (Figs. 4 and 5). Furthermore, the structure of intercellular

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EXPLANATION OF FIGURE 6

Above: Photomicrograph of a lesion, three weeks old, produced by freezing the wall of the lower part of the abdominal aorta, together with a small branch, in a rabbit with an average blood cholesterol value of 620 mg. per 100 cc. In this animal, 18% of the intima of the thoracic aorta was involved by atheromas, and there were no microscopic spontaneous atheromas of the lower part of the abdominal aorta. The horizontal and vertical letters indicate the extent of the proliferated intima (*I*), the degenerate media (*M*), and the adventitia (*A*) of the aorta and the lumbar branch, respectively. The arrow points toward the origin of the lumbar branch. Note that the proliferated intima of the aorta and its small branch is continuous and only very slightly modified, especially on the aortic side, by accumulation of lipids. Note also that there is a sharp contrast between the degenerate media of the aorta, which is free from lipids, and the degenerate media of the lumbar branch, which is highly vacuolated with lipid deposits. This medial localization is characteristic in small muscular arteries.

Below: Photomicrograph of a lesion, 28 weeks old, produced by freezing the wall of the renal artery in a rabbit with an average blood cholesterol value of 1,190 mg. per 100 cc. In this animal, 12% of the intima of the thoracic aorta was involved by atheromas, and there were no microscopic spontaneous atheromas of the renal artery. The extent of the proliferated intima (*I*), the degenerate media (*M*), and the adventitia (*A*) of the renal artery is indicated. Note the severe atheromatous localization in the regenerated intima and the degenerate media. The internal elastic membrane is defective over a large area. Despite the severe medial atheromatosis, the adventitia, as always, remains uninvolved by the process.

materials in the new intima was often modified. The specific evidence of atheromatous localization was variable in quantity and location. Ordinarily, sheets or scattered clusters of lipophages were found at all levels in the proliferating intima (Fig. 4). At times, however, the lipophages were located only in the superficial or the deep layers. Occasionally they were well covered by a thick layer of proliferating intima (Fig. 5, left).

In addition to the specific evidence of atheromatosis in the form of clusters of lipophages, there was presumptive evidence in the form of modifications of newly formed fibroelastic tissue in the intima (Fig. 5). These modifications, never encountered in our experience with intimal proliferation in normocholesteremic animals, occurred in two forms. One was in the nature of a loosely reticular mucinous type of fibrillar collagenous structure (Fig. 5, left). This resembled the "mucinous degeneration" of collagen often found in human arteriosclerosis. The other and more frequent modification was a homogeneous hyaline fusion of collagenous fibrils (Figs. 5, right, and 7). This resembled the "hyaline degeneration" of collagen encountered in human arteriosclerosis. These two changes in the intercellular matrix of the proliferated intima frequently occurred together in the same lesion. They were especially conspicuous in old animals, "hyaline" degeneration being prominent in old lesions, while "mucinous" degeneration often occurred alone in lesions less than nine weeks old. Though lipophages were often distributed at random throughout lesions showing "mucinous" and "hyaline" degenerative changes, they were occasionally absent.

Previous studies showed that local lesions produced by freezing the iliac or the renal artery of a normocholesteremic animal were similar in most respects to those produced by freezing the aorta. A new vascular wall was formed in the framework of the intima which proliferated at the site of medial degeneration. Twenty-one lesions produced by freezing the iliac artery in hypercholesteremic animals were studied in serial sections. Localization of lipids occurred in the proliferating intima in 17 lesions. In one lesion there was presumptive evidence of atheromatous localization. In three lesions there was no evidence of atheromatosis. The localization had no specific correlation with the presence of atheromas, rarely found just above or below the level of the lesion. There was less evidence of intimal proliferation being inhibited by atheromatous localization in the iliac arterial lesions than in the aortic lesions. The principal evidence of atheromatous localization in iliac arterial lesions was in the form of lipophages usually occurring in clusters at various levels in the proliferating intima. "Mucinous" and "hyaline" degenerative changes of the collagen of the proliferated intima were conspicuous in only three lesions. There were, however, large aggregates of lipophages in the degenerate media in five lesions. The presence of these cells in the media was correlated with the presence of discontinuities in the internal or the external elastic membrane. The atheromatous changes in the media were not always adjacent to or contiguous with similar changes in the intima. It was apparent that here we were dealing with a form of medial vascular disease which did not occur either in aortic lesions or in the media of the iliac artery elsewhere than at the site of degeneration produced by freezing.

Seventeen lesions produced by freezing the renal artery were studied in serial sections. Fourteen lesions showed definite evidence of atheromatous localization (Figs. 6, lower part, and 7). One showed presumptive evidence. Two lesions were

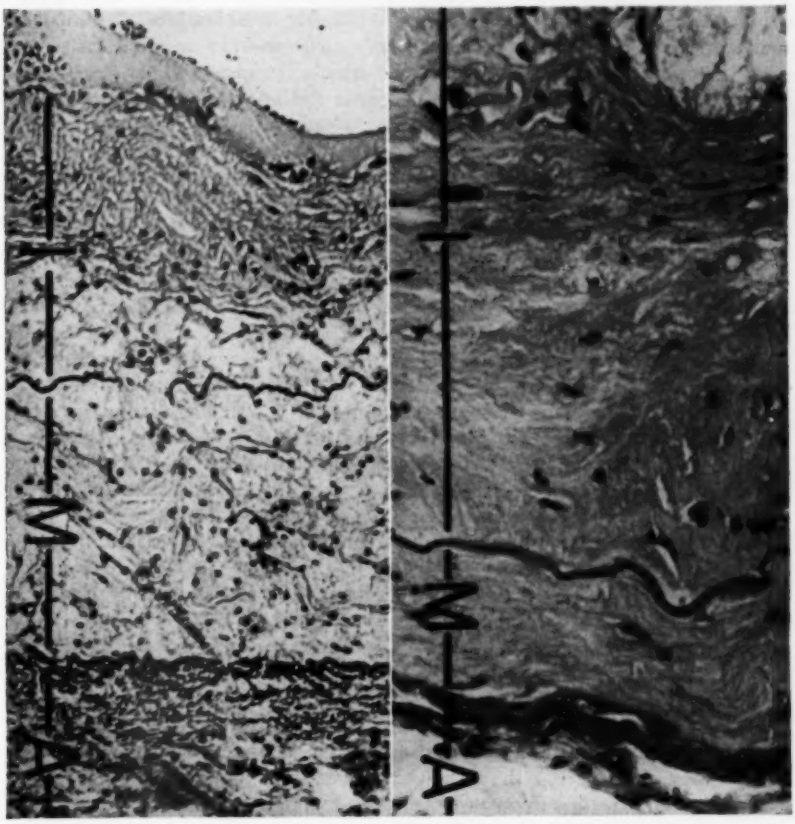


Fig. 7.—Left: High-power photomicrograph of the wall of the renal artery shown in Figure 6, lower part. Note the infiltration of lipophages in the inner one-half of the thickened intima (*I*). The entire thickness of the media (*M*) is occupied by masses of similar lipophages. The internal elastic membrane is discontinuous. The external elastic membrane and adventitia (*A*) are normal. Note the difference between the newly formed abnormal collagen in the superficial intima and the normal collagen of the adventitia.

Right: Photomicrograph of a lesion, 52 weeks old, produced by freezing the wall of the renal artery in a rabbit with an average blood cholesterol value of 560 mg. per 100 cc. In this animal, 76% of the intima of the thoracic aorta was involved by atheromas, and there were no microscopic spontaneous atheromas of the renal artery. A part of the proliferated intima (*I*), the full-thickness of the degenerate media (*M*), and the adventitia (*A*) are shown. This shows localization of lipids and lipophages in the proliferated intima. Beneath the lipid deposits there are a few residual degenerating fibrocytes surrounded by fused bundles of "hyalinized" collagenous fibrils. Similar changes are conspicuous in the collagen which has been deposited in the repair of the degenerate media. These changes in collagen seem to follow the slow resorption of lipids deposited in the intimal and medial tissues, especially of old animals.

free from changes attributable to atheromatosis. The atheromatous changes occurred either only in the media or in the proliferated intima and the degenerate media. The proliferated intima was never exclusively involved, but in six instances all atheromatous changes were restricted to the degenerate media beneath the thick fibroelastic layer of proliferating intima. The specific evidence of atheromatous localization was in the form of masses or sheets of lipophages in the intima of eight lesions and in the degenerate media of 14 lesions (Figs. 6, lower part, and 7). Presumptive evidence of atheromatous localization was in the form of a "waxy, mucinous, or hyaline" change of the stroma of the thickened intima or the degenerate media. These changes occurred alone in only one artery but were found in conjunction with lipophages in lesions of five arteries (Fig. 7).

The amount and the type of atheromatous and stromal degenerative changes were similar in iliac and renal arterial lesions of the same animal. The principal difference was in the preponderant medial localization in the renal artery. This was regularly accompanied by discontinuities in the internal or the external elastic membrane (Fig. 6, lower part, and Fig. 7, left). In the presence of discontinuities of the internal elastic membrane it was usually possible to find that the atheromatous deposit in the proliferated intima was continuous with lipid deposits in the inner part of the degenerate media. However, the extent of the lipophage reaction was always much greater around the circumference of the vessel within the media than the extent of the defect of the structural continuity of the internal elastic membrane (Fig. 6, lower part). In several instances the defects seemed to be exclusively in the external elastic membrane and the degenerate media was vascularized by capillaries penetrating into it from the adventitia. This defectiveness was not accompanied by a lipophage reaction in the adventitia. The lipophages were localized in the media. *These facts led to the conclusion that we were dealing here, as in the case of iliac arteries, with a form of medial arterial atheromatosis which was not necessarily connected with proliferative intimal atheromatous disease.* Similar medial atheromatous changes were found in lesions of small arterial branches of the aorta where unintentional damage had been done during the process of freezing the aorta. Here, it was possible to compare in the same microscopic field a minute muscular artery near its point of origin from the aorta with the latter and to note the distinct differences between the medial reactions of the two (Fig. 6, upper part).

## COMMENT

The diet fed in this experiment resulted in hypercholesteremia, which varied from animal to animal and from time to time for unknown reasons. Young animals tolerated the diet better than old ones. They gained some weight but at less than the normal rate. The old animals usually lost weight, and this became rather acutely conspicuous after several months of the diet.

Previous experience had shown that animals restricted to this diet ordinarily began to accumulate intimal atheromatous deposits in the proximal part of the aorta after about 22 weeks. More prolonged feeding usually resulted in maintenance of elevated cholesterol levels or in yet higher elevation of the blood cholesterol. This was accompanied by a gradual increase in the amount of aortic intimal atherosclerosis, with the intimal involvement progressing distally toward and beyond the origin of the renal arteries. There was a simultaneous but variable increment in intimal atherosclerosis of some major arterial branches.

The form of vascular disease encountered in previous experiments and in the present series of animals was similar to that commonly observed in rabbits fed different types of diets rich in cholesterol.[4] Plaques consisting of lipophages developed in the intima (Fig. 1). These enlarged at variable rates and often became confluent (Fig. 2). Later, the lipophage structure of plaques often became indistinct. A web-like reticular structure developed, and within the framework of this structure the lipid deposits, frequently in crystalline form, persisted (Fig. 3). Fibroblastic organization of plaques with conspicuous deposition of collagen or other changes, such as "mucinous" or "hyaline" degeneration of the meager stroma of plaques, was rarely recognized. Nor was there any important structural change in the media of the aorta for any significant distance beneath the plaques (Fig. 2). The media of other arteries, especially the coronary and gastroduodenal vessels, however, often showed degenerative changes opposite the plaques of severe intimal atherosclerosis (Fig. 1).

These observations led to three conclusions. First, the hypercholesteremic atherosclerosis of young and old rabbits resembled human senile arteriosclerosis in that it occurred initially as a local disease of the intima. Second, it developed, as does the human counterpart, as a segmental disease of particular arterial systems. Third, the magnitude of the disease of a particular arterial system, as in human arteriosclerosis, had no constant correlation with the magnitude of aortic disease or of atherosclerosis of other arterial systems. Finally, the hypercholesteremic atherosclerosis of rabbits differed from human senile arteriosclerosis in several important particulars. Though the lipophage plaque was closely similar to plaques found in human atherosclerosis, it differed from that encountered in human disease in that it commonly evolved into a web-like structure (Fig. 3). In human disease, the proliferative reactions of the intima with simulated formation of new vascular walls in fibrous or fibroelastic frameworks of proliferated intima are conspicuous features. This is often accompanied by intimal or medial degenerative changes with "hyalinization," "mucinous" degeneration, and calcification, more or less complicated by atheromatous deposits.

It was believed that some differences between hypercholesteremic atherosclerosis of rabbits and human atheroarteriosclerosis might be investigated by producing experimentally local medial degeneration and reactive intimal proliferation in animals in a hypercholesteremic state suitable for the depositing of lipids in "normal" intima. Previous experiments had shown that local freezing of the vascular wall was a good method of obtaining a reproducible form of medial degeneration.[1] This method did not disturb the general continuity of the vascular wall and was advantageous because of the negligible transient inflammatory reaction following freezing. Indeed, vascularization of the degenerate aortic media rarely occurred even though cells were destroyed in all layers of the vascular wall (Fig. 4). The reactive proliferation of the aortic intima was prompt and proceeded over a period of several weeks to form a thick fibroelastic framework in which structure gradually reoriented in a pattern closely resembling that of the original vascular wall (Fig. 6, upper part). This pattern of reaction had many features in common with intimal reactions occurring with or without atheromatous deposition in human arteriosclerosis. The intimal reactions of normocholesteremic old animals differed from those of young animals in only minor respects. The intimal proliferation had somewhat less magnitude and was more delayed in old animals. "Hyaline" and "mucinous" degeneration was not encountered.

The principal purpose of the present experiments was to study the development of the fibroelastic form of intimal proliferation at sites of medial degeneration produced by freezing the vascular walls of young and old hypercholesteremic animals in which intimal atheromas were known to be developing. In general, experience had shown that under the experimental dietary conditions atheromatous plaques became grossly apparent in the proximal aorta after about 22 weeks. Therefore, 11 old rabbits and 12 young rabbits were operated upon at this time. Lesions were produced by local freezing of the distal aorta, one iliac artery, and one renal artery. A complete microscopic study was made of the lesions and of appropriate control segments of the vascular tree at intervals of time up to 52 weeks after production of the lesions.

These studies led to three general conclusions: First, the zones of intimal proliferation usually proved to be elective sites for accumulation of lipids (Figs. 4 and 5). Second, the zones of medial degeneration of the iliac and especially of the renal artery also were elective sites for accumulation of lipids (Figs. 6, lower part, and 7). Third, the accumulation of lipids in the proliferated intima and degenerate media was often accompanied by conspicuous modifications in the microscopic appearance of the reparative process (Figs. 4, 5, and 7).

The accumulation of lipids in zones of intimal proliferation was neither uniform nor homogeneous. There was an admixture of lipophages, fibrocytes, smooth muscle cells, and stromal elements with an excess of one or the other at various levels in the newly formed intima (Figs. 4, 5, and 7). Though the pattern of their relationship varied from one animal to another, it was similar in different lesions of the same animal. However, intimal accumulation of lipids was generally more pronounced in aortic than in arterial lesions. There was no reliable correlation between the accumulation of lipophages in proliferated intima and the atheromatous changes in the intima of the vascular segment above or that below the lesion. In all except five animals the intimal changes above and below aortic lesions consisted of microscopic miliary scattered mound-like reticular or lipophage plaques. The intimal changes proximal and distal to the arterial lesions were of still lesser significance, especially in the renal arteries.

The accumulation of lipids in zones of medial degeneration was often conspicuous. It was occasionally encountered in the degenerate media of aortic lesions (Fig. 2). Here, however, the lipophage infiltration was always restricted. Usually, the depth of infiltration did not exceed the limits of the innermost two or three elastic lamellae. The outer two-thirds of the degenerate media was never involved. Lipid accumulation in macrophages in the degenerate media of arterial walls was often conspicuous (Figs. 6 and 7, left). In several lesions, especially of the renal arteries, the entire thickness of the media was composed of lipophages and other signs of atheromatous accumulation (Fig. 6, lower part, and Fig. 7, left). At times the medial atheromatosis far exceeded the intimal atheromatosis, and no connection between the two processes could be distinguished. At other times the atheromatous changes in the proliferated intima and degenerate media were continuous with one another through discontinuities in the internal elastic membrane (Fig. 6, lower part, and Fig. 7, left). Frequently, the discontinuities were small, and it was not possible to be certain that they represented channels through which lipids passed from the intima into the media. This was especially true in lesions in which there were no demonstrable lipids in the

intima at levels adjacent to severe medial atheromatosis. In these cases, it was possible that discontinuities in the external elastic membrane were the channels through which lipids, along with a vascularizing capillary network, penetrated the media. But even here there was no accumulation of lipophages or lipids in the adventitia. The disease was confined to the zone formerly occupied by smooth muscle of the vascular wall (Figs. 6 and 7, left). A simple mechanical explanation for this interesting form of medial atheromatosis is not quite sufficient, but we have no better explanation to offer. Some results were in accord with the conclusions reached by Duff, who stated that "the lipid deposits are really an index of injury to the walls of the vessels."[6] This was, however, not always the case.

The third conclusion from these studies was that the reparative processes at the sites of lesions produced by freezing were modified by atheromatous accumulations. In the first place, the amount of intimal proliferation was reduced in sites where atheromatous deposits occurred (Figs. 4 and 5). Secondly, the usual perfect pattern of fibroelastic intimal proliferation was distorted by the process of atheromatous deposition (Figs. 4 and 5). Third, several forms of "degenerative" changes occurred in the fibroelastic stroma of the proliferated intima (Figs. 4, 5, and 7). These degenerative changes usually resembled changes ordinarily called "hyaline" or "mucinous" degeneration. The collagen was principally affected, although elastic fibrils, fibrocytes, and smooth muscle cells in the newly formed intima were also implicated. These changes were most conspicuous in old animals and were not always spatially related to recognizable atheromatous deposits. They were more common in the proliferated intima but were occasionally conspicuous in the stroma of medial lesions involved by atheromatous deposits (Fig. 7, right). It seemed that the presence of lipids had in some way modified the nature of the newly formed collagen and, perhaps indirectly, the elastic tissue. Simultaneously, the proliferating and mature cells of the intima were adversely affected.

In any event, the complex pattern of atheroarteriosclerosis encountered in these lesions, as described, bore a closer resemblance to the varied types of human atheroarteriosclerosis than did the simple intimal atheromas occurring in hypercholesteremic rabbits. It was concluded that the pathogenesis and the pattern of evolution of the varied forms of human senile vascular disease may be more accurately studied in the experimental animal by taking cognizance of at least three factors: medial degeneration, fibroelastic intimal proliferation, and altered composition of the lipids of the blood. These factors may occur singly or together, and in each instance a different form of vascular disease develops.

## SUMMARY

Eleven old rabbits and 12 young rabbits were maintained on a diet rich in cholesterol. After 22 weeks, at the time when intimal atheromatous plaques were forming in the animals, focal lesions of the aorta, the renal artery, and the iliac artery were produced by freezing the vascular walls. The sequences of degeneration and regeneration were studied at the sites of freezing in serial microscopic sections of lesions varying from 2 to 52 weeks in age. These studies showed that atheromatous

6. Duff, G. L.: The Nature of Experimental Cholesterol Arteriosclerosis in the Rabbit, Arch. Path. 22:161 (Aug.) 1936.

localization occurred in many lesions and to the same degree in young and old animals. The localization was more conspicuous in the fibroelastic plaques of regenerated intima of the aorta than in similar plaques which formed in the iliac or the renal arteries. The localization in the degenerate media was conspicuous only in arterial walls, more so in the renal than in the iliac arteries. The accumulation of lipids in the lesions was accompanied by inhibition of the processes of repair and distortion of the usual pattern of structural arrangement, especially of regenerating intima. Furthermore, "hyaline" and "mucinous" degenerative changes of the new connective tissue stroma frequently occurred in association with atheromatous deposits, especially in the regenerated intima of old animals. This combination of complex vascular disease involving active medial degeneration, fibroelastic intimal regeneration, and atheromatous localization simulated the varied aspects of human senile atheroarteriosclerosis much more closely than did the simple atheromatous intimal disease of hypercholesteremic rabbits.

# CERTAIN FEATURES OF THE VASCULAR BEDS OF THE CORTICO-MEDULLARY AND MEDULLARY REGIONS OF THE KIDNEY

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THE VASCULAR bed of the kidney and the intrarenal distribution of blood have received much attention in recent years. In the course of studies related to the renal vasculature we have been impressed by three frequent observations, namely (1) that renal vasoconstriction appears to be limited to the cortex, (2) that under conditions of sharply decreased blood flow and pressure in the kidney the medulla frequently appears congested even though blood is not flowing through it, and (3) that under conditions of such decreased blood flow and pressure to the kidney the blood flow is best preserved through the corticomedullary portion of the kidney.

## CORTICAL RESTRICTION OF RENAL VASOCONSTRICTION

That renal vasoconstriction is primarily restricted to the cortex has been observed in the following conditions.

*During Electric Stimulation of the Renal Nerves.*—With the onset of stimulation of the renal nerves under optimal conditions, the kidney of the rat, the rabbit, or the dog blanches grossly, but when carefully studied on cross section the medulla remains red in contrast to the pale cortex. Yet, India ink injected intravascularly fails to appear in the blanched kidney even though the medullary vessels are filled with blood. The same findings have been observed in the rabbit initially with stimulation of the sciatic nerve.

*After Sudden Occlusion of the Renal Nerves.*—After sudden clamping of the renal artery of the rat, the rabbit, or the dog, the kidney blanches. On cross section, the cortex is pale while the medulla remains red.

*After Intravascular Injection of Substances Producing Renal Vasoconstriction.*—Within a few seconds after epinephrine hydrochloride (0.1 cc. of 1 : 1,000,000 solution), vasopressin (0.001 unit), protamine (5 to 10 mg.), or hemolyzed homologous stroma-free red cells have been injected intravascularly into the rat, the findings are the same as those during electric stimulation of the renal nerves and after sudden occlusion of the renal artery.

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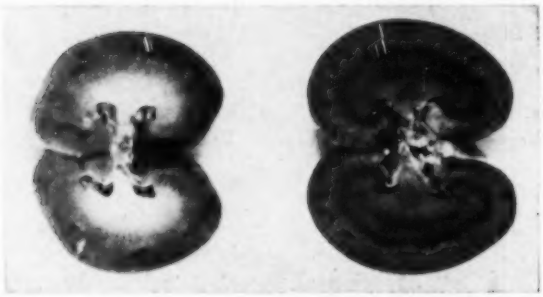

Fig. 1.—Congestion of the renal medulla after release of a clamp occluding the renal artery. The artery to the kidney shown on the right was completely occluded for two hours, and 24 hours later both kidneys of this rat were removed. Especially marked congestion is evident in the medulla of the kidney on the right.

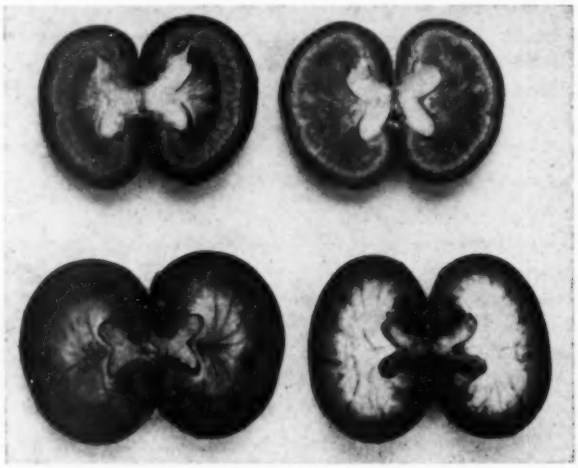

Fig. 2.—Deviation of blood from the renal medulla. The upper pair of rabbit kidneys illustrates the normal appearance of the cross section of the kidney after the intravascular injection of India ink.

The lower pair of kidneys was removed from another rabbit after the intravascular injection of India ink during continuous stimulation of the left sciatic nerve. Both kidneys had blanched initially with the onset of stimulation. The kidney on the right, removed two minutes after the onset of stimulation, shows the presence of ink only in the cortex and corticomedullary regions. Ink appeared in the renal vein before removal of this kidney. The kidney shown on the left, removed four minutes after the onset of stimulation, shows a more complete distribution of ink throughout the kidney.

### CONGESTION OF THE RENAL MEDULLA

That the medulla of the kidney may be congested and yet no blood flow through it has been observed in the following conditions.

*After Release of a Clamp Occluding the Renal Artery.*—After the removal of a rubber or polythene-shod clamp which has completely occluded the renal artery for two to four hours in the rat or the dog, the medulla frequently appears severely congested (Fig. 1). However, examination of the kidney removed after an intravascular injection of India ink often shows that the ink has passed through the cortex, including the corticomedullary region, but little or no ink is present in the congested medulla. This finding has been observed even 24 hours after removal of the clamp occluding the renal artery.

*During Severe Constriction of the Renal Artery.*—After severe constriction of the left renal artery of the aorta between the origin of the renal arteries (above the left one) in the rat, the cross section of the left kidney often reveals a congested medulla. However, after the intravascular injection of India ink, the ink is found in the cortex, including the corticomedullary region, but not in the congested medulla.

*After Severe Renal Cortical Vasoconstriction Produced by Stimulation of the Renal or the Sciatic Nerves.*—Electric stimulation of the renal nerves or of a sciatic nerve to produce maximal effects in the rat or the rabbit produces definite renal cortical vasoconstriction which is sufficient to stop renal blood flow for one to two minutes. However, in spite of continuous stimulation, blood flow returns through the kidney as the vessels relax. During this brief period of recovery of blood flow through the kidney, India ink injected intravascularly sometimes is found throughout the cortex, including the corticomedullary region, but little or no ink is present in the medulla (Fig. 2).

*During Prolonged Vascular Hypotension.*—India ink injected intravascularly in a dog whose mean arterial blood pressure has been maintained at about 70 mm. of mercury by controlled hemorrhage for six hours or longer is found sometimes in the cortex and corticomedullary regions of the kidney but is absent in the congested medulla.

### PRESERVATION OF BLOOD FLOW IN THE CORTICOMEDULLARY REGION

Evidence that renal blood flow is best preserved in the corticomedullary region has been observed in the following conditions.

*After Prolonged Vascular Hypotension.*—After the mean arterial blood pressure of dogs has been maintained at about 70 mm. of mercury by controlled hemorrhage for 6 to 26 hours, severe degenerative tubular lesions are observed in the kidneys.[1] The degenerative changes frequently are much less severe in the corticomedullary region than in the remainder of the cortex. In several cases the cortex above and the medulla below the corticomedullary region are completely necrotic but the corticomedullary region shows only mild to moderate degenerative changes.

*After Constriction of the Renal Artery.*—Kidneys of the rat and the dog removed several days after constriction of the renal artery have occasionally contained infarcts in the peripheral parts of the cortex with moderate or no microscopic evidence of damage to the lower cortex and corticomedullary tissue.

1. Block, M. A.; Wakim, K. G.; Mann, F. C., and Bennett, W. A.: Unpublished data.

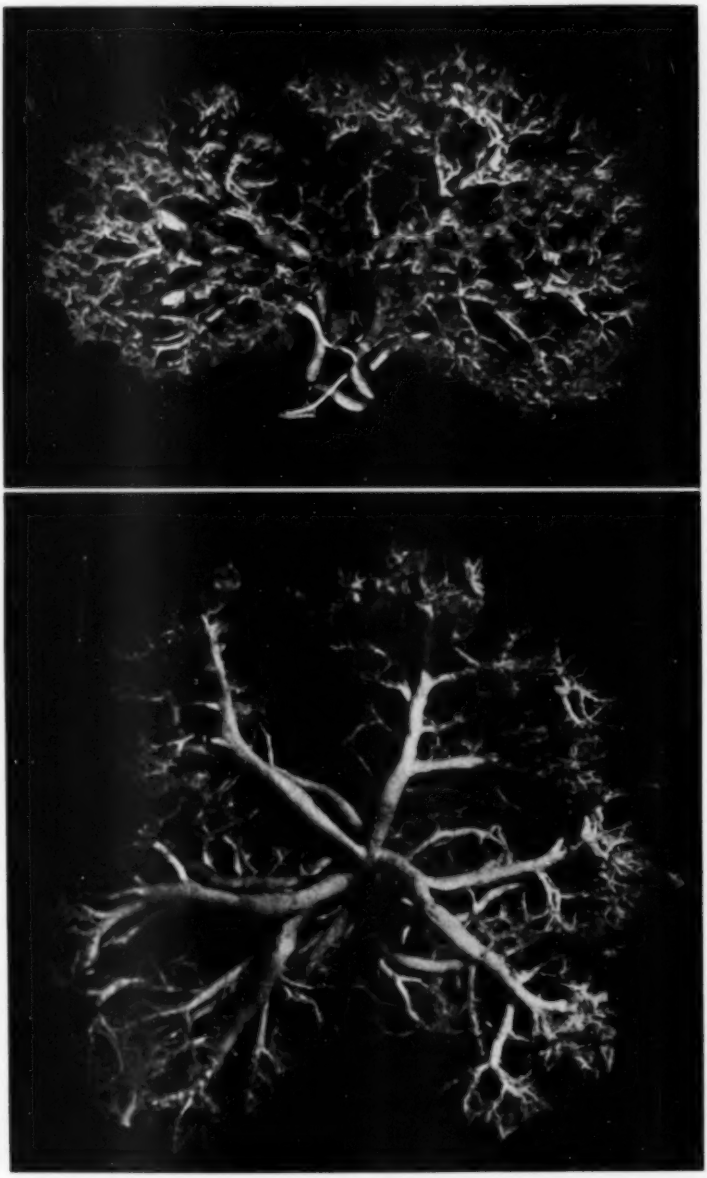

Fig. 3.—Neoprene casts of the renal vascular tree illustrating primarily perfusion of the corticomedullary region. Of the two kidneys shown on top, the right illustrates a fairly

*(Legend continued on next page)*

Figure 3.—Continued

complete cast of the vascular bed of the renal cortex while the left shows a cast chiefly of the vessels in the corticomedullary region. One may note the presence of both arteries and veins, running side by side. The lower pictures show sections of the same kidney spread out.

*Neoprene Casts of the Renal Vascular Bed.*—Neoprene injected into the renal artery or the aorta in vivo produces severe renal vasoconstriction which results in a very incomplete cast of the vascular bed of the kidney. After digestion of the renal parenchyma, a cast of only the corticomedullary region of the kidney, including both the arterial and the venous bed of this region, is often found (Fig. 3). Evidently in such cases the vasoconstriction peripheral to the corticomedullary region is sufficient to prevent neoprene from passing through any renal tissue other than the corticomedullary area.

## COMMENT

The vascular bed of the renal medulla reacts differently and is structurally different from that of the renal cortex. This statement is supported by the above observations and the morphologic studies reported by others.[2]

Our studies indicate that a pale renal cortex and a medulla filled with blood are not evidence that blood was flowing only or largely through the medulla of the kidney. Also, blood flow may be largely restricted to the cortex even though the medulla is congested. However, cortical tissue is more susceptible to ischemia than is medullary tissue. Since these deviations in the intrarenal distribution of blood occur in conditions of a severe decrease of pressure and flow of blood into the kidney, they apparently are not of great importance in localization of injury of renal tissue between cortex and medulla. The degree of ischemia may be great enough to produce renal injury most severe in the cortex even though blood flow may be largely restricted to the cortex.

Other investigators have also observed that under conditions of decreased blood pressure and blood flow to the kidney, the medulla is poorly perfused and blood flow is best preserved through the corticomedullary region.[3] The presence of medullary congestion and the corticomedullary localization of the least severe degeneration have been observed in human kidneys after shock.[4] We have obtained this same result with prolonged experimental hypotension in dogs.[1] Hellerstein, Orbison, Rodbard, Wilburne, and Katz [5] observed in experimental malignant hypertension the occurrence of severe degeneration in the kidney, but the corticomedullary region was spared.

Blood normally flows through the renal medulla, but since our observations indicate that blood can flow through the corticomedullary region without passing through the medulla, it is apparent that there are two possible vascular pathways which blood leaving the corticomedullary glomeruli may take. The blood may flow to the venous channels without first flowing through the medulla, or it may flow through the medulla and then pass into the veins. Morphologically, these two variations in blood flow in the corticomedullary region are possible. The efferent

2. Trueta, J.; Barclay, A. E.; Daniel, P. M.; Franklin, K. J., and Prichard, M. M. L.: Studies of the Renal Circulation, Springfield, Ill., Charles C Thomas, Publisher, 1947.

3. (*a*) Schlegel, J. U., and Moses, J. B.: Proc. Soc. Exper. Biol. & Med. **74**:832, 1950. (*b*) Kahn, J. R.; Skeggs, L. T., and Shumway, N. P.: Circulation **1**:445, 1950. (*c*) Insull W., Jr.; Tillotson, I. G., and Hayman, J. M., Jr.: Am. J. Physiol. **163**:676, 1950. (*d*) Heggie, J. F.: Lancet **2**:654, 1947. Trueta and others.[2]

4. Darmady, E. M.: Brit. J. Surg. **34**:262, 1947. Maegraith, B. G., and Findlay, G. M.: Lancet **2**:403, 1944. McManus, J. F. A.: Medical Diseases of the Kidney, Philadelphia, Lea & Febiger, 1950.

5. Hellerstein, H. K.; Orbison, J. L.; Rodbard, S.; Wilburne, M., and Katz, L. N.: Am. Heart J. **42**:271, 1951.

arterioles of the corticomedullary glomeruli divide into two types of vessels, namely, arteriolae rectae spuriae, which supply the medulla, and peritubular capillaries, which supply the corticomedullary region only. Normally, blood flows through both vessels, but our observations and those of other investigators suggest that when renal blood flow is severely reduced most of the blood leaving the corticomedullary glomeruli frequently passes through the peritubular capillaries in the corticomedullary region, with little or no blood flowing through the arteriolae rectae spuriae. However, the medullary vessels frequently remain filled with static blood under such conditions.

We wish to emphasize that not all of the patterns of intrarenal blood flow presented herein are constantly present under the conditions cited. Other variations in the intrarenal distribution of blood flow also occur. However, the patterns herein described occur frequently enough to warrant the conclusions made concerning the corticomedullary and medullary vascular beds.

## SUMMARY AND CONCLUSIONS

1. Vasoconstriction of the medullary vascular bed of the kidney does not seem to be produced by conditions which produce cortical vasoconstriction. However, the medulla is frequently congested even though the flow of blood in the kidney has stopped. The blood in the medullary vessels is static.

2. The presence of medullary congestion does not necessarily indicate that blood is flowing through the medulla. In fact, little or no blood may flow through a congested medulla.

3. When blood pressure and flow to or within the kidney are markedly reduced, the renal tissue last to be supplied with blood (peripheral cortex and medulla) may receive little or no blood. Blood flow is best preserved through the corticomedullary region under these conditions.

4. It is probable that under conditions of diminished blood flow and pressure the blood leaving the corticomedullary glomeruli sometimes flows only through the peritubular capillaries in the corticomedullary region without flowing through the arteriolae rectae spuriae into the medulla.

# A POSTMORTEM STUDY OF VASCULAR DISEASE IN DIABETICS

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SINCE the discovery of insulin and the antibiotics a majority of patients with diabetes are protected against death from diabetic acidosis and infections. They survive much longer than formerly, but a larger percentage die of vascular lesions. The accelerating influence of diabetes upon atherosclerosis has been known a long time. The purpose of this paper is to make a quantitative determination of this influence by comparing the incidence of deaths from vascular disease in diabetics and nondiabetics. The various factors which may influence the development of atherosclerosis will also be discussed.

The material available for study consists of 1,559 autopsies performed on diabetic subjects at the University of Minnesota from January, 1910, through May, 1951. The clinical records and autopsy protocols were studied, and histological material was available in over 75%. The distribution of the autopsies was as follows: 126 in the period from 1910 to 1929; 342 in that from 1930 to 1939; 1,091 in that from 1940 to 1951. Thus 70% were made after 1939, and less than 5% belong to the pre-insulin era. The subjects were all residents of the cities of Minneapolis and St. Paul, and the treatment they received was probably as good as the average throughout the country during this period.

## RESULTS

*Age at Onset* (Table 1).—The onset of the disease is dated from the first appearance of diabetic symptoms or from the first finding of sugar in the urine. In Table 1 a group is shown for whom the exact time of onset was unknown, being indicated in the clinical records as a "few," "several," or "many" years. These are tabulated according to the probable decade of onset. This is admittedly unsatisfactory, but if these cases were omitted from the table, it would cause a false increase in the proportion of cases beginning in the first four decades.

These data are at best only approximate, since it is obvious that the first noting of glycosuria in a case of mild diabetes does not date the onset of the disease. In many of the older diabetics of this group the disease was first recognized when the patient was admitted to the hospital because of impending gangrene or coronary disease. Sometimes sugar was found in the urine in the routine examination of an asymptomatic patient. According to Millard and Root,[1] mild diabetes may be present months or years before it is recognized. Numerous diabetic surveys have shown

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This study was aided by a grant from the United States Public Health Service.

1. Millard, E. B., and Root, H. F.: Am. J. Digest. Dis. **15**:41, 1948.

that many persons with mild diabetes are unaware of any illness. The date of onset is probably more accurately determined for the younger groups than it is for the older, since mild diabetes occurs in more of the older persons.

It appears from Table 1 that diabetes developed before the age of 20 years in about 5% of the cases and before the age of 40 years in about 16%. In about 70% it developed after the age of 50 years. A few cases are first recognized in the ninth decade. Extensive data on age incidence are given by Pincus, Joslin, and White.[2]

TABLE 1.—*Age of Onset of Diabetes*

| Age of Onset, Yr. | Number of Patients | Number for Whom Date of Onset Is Definite | Number for Whom Date of Onset Is Inexact | % of Total Group |
|---|---|---|---|---|
| 0-10 | 21 | 21 | 0 | 1.3 |
| 10-20 | 60 | 56 | 4 | 3.8 |
| 20-30 | 77 | 67 | 10 | 5.0 |
| 30-40 | 97 | 93 | 4 | 6.2 |
| 40-50 | 230 | 203 | 27 | 14.8 |
| 50-60 | 414 | 348 | 66 | 26.5 |
| 60-70 | 404 | 329 | 75 | 25.9 |
| 70-80 | 209 | 160 | 49 | 13.4 |
| 80-90 | 47 | 37 | 10 | 3.0 |
| Total | 1,559 | 1,314 | 245 | 99.9 |

TABLE 2.—*Length of Life of Diabetics with Respect to Age of Onset*

| Age at Onset, Yr. | Total No. of Patients | Number with Stated Duration of Life After Onset | | | | |
|---|---|---|---|---|---|---|
| | | 0-10 Yr. | 10-20 Yr. | 20-30 Yr. | 30-42 Yr. | Indefinite |
| 0-10 | 21 | 13 | 4 | 4 | .. | .. |
| 10-20 | 60 | 25 | 20 | 10 | 1 | 4 |
| 20-30 | 77 | 38 | 18 | 10 | 1 | 10 |
| 30-40 | 97 | 47 | 28 | 14 | 4 | 4 |
| 40-50 | 230 | 113 | 62 | 22 | 4 | 29 |
| 50-60 | 414 | 234 | 96 | 18 | — | 66 |
| 60-70 | 404 | 283 | 41 | 5 | — | 75 |
| 70-80 | 209 | 153 | 7 | — | — | 49 |
| 80-91 | 47 | 37 | — | — | — | 10 |
| Total | 1,559 | 943 (60.5) % | 276 (17.7) % | 83 (5.3) % | 10 (0.6) % | 247 (15.8) % |
| 0-40 | 255 | 123 (48.2) | 70 (27.7) | 38 (14.9) | 6 (2.3) | 18 (7.0) |
| 40-60 | 644 | 347 (53.9) | 158 (24.5) | 40 (6.4) | 4 (0.6) | 95 (14.7) |
| 60-91 | 660 | 473 (71.7) | 48 (7.3) | 5 (0.7) | 0 (0.0) | 134 (20.3) |

The earliest onset of the disease in this series was at the age of 16 months. Limper and Miller [3] collected 34 cases having onset during the first year of life. In one instance the disease was recognized at the age of 9 days. In the oldest patient of our series diabetes was first recognized at the age of 91 years.

*Length of the Diabetic Life.*—The length of life is shown with respect to the age of onset in Table 2. It is clear that much better results could have been accomplished, especially with the younger diabetics; but this is a record of what actually occurred. If the 126 subjects who died prior to 1930 are excluded, the picture is slightly less gloomy.

2. Pincus, G.; Joslin, E. P., and White, P.: Am. J. M. Sc. **188**:116, 1934.

3. Limper, M. A., and Miller, A. J.: Diabetes Mellitus with Extensive Gangrene in Early Infancy, Am. J. Dis. Child. **50**:1216 (Nov.) 1935.

There were 21 subjects whose disease began in the first decade (0 to 10 years). Four infants, aged 16 months to 2 years, were poorly controlled, and all died within three months. Four children with onset from 3 to 4 years of age survived two to six years. Three children with onset at age 5 survived 6, 21, and 23 years, respectively. Eight of the 21 lived over 10 years, and four lived over 20 years. All were dead within 23 years of onset, and the oldest survivor was 30 years of age.

Of 60 persons with onset in the second decade (10 to 20 years), 8 died during the first year of the disease and 6 died from one to five years after the onset. Thirty-one lived more than 10 years, and 10 from 20 to 30 years. One man whose disease began at the age of 18 years died at age 60 after 42 years of diabetes. This is the longest period of survival in our records.

Of 77 juvenile diabetics (onset before the age of 20 years) the duration of whose disease was known, 39 lived more than 10 years and 15 more than 20 years. This appears to be a lower percentage of long-term survivors than is generally reported, but I cannot find reports in the literature entirely comparable to mine in which every case is included.

Of the 77 subjects with onset in the third decade (20 to 30 years), 10 died during the first year and 18 from one to five years after onset. Twenty-nine lived beyond 10 years, and eleven beyond 20 years. One man with onset at the age of 28 years died at the age of 68 years after 40 years of diabetes.

There were 97 persons in whom the disease began in the fourth decade (30 to 40 years). Thirteen of these died during the first year of the illness, and 21 others died within five years. Forty-six persons lived more than 10 years, 18 more than 20 years, and 4 more than 30 years. One person survived 40 years of diabetes.

Ten of 230 subjects with onset in the fifth decade (40 to 50 years) died within one week, having been admitted in diabetic coma. Eleven others died within one year, and 61 were dead within five years. Eighty-eight of the group lived more than 10 years, twenty-six more than 20 years, and four more than 30 years. The longest period of survival was 35 years.

In the group with onset in the sixth decade (50 to 60 years) 18 persons lived about one week after diabetes was diagnosed. On admission they were suffering from pyemia, coma, or some vascular lesion. It is highly probable that diabetes had been present a long time. Fifty-five patients died during the first year of the disease and 142 persons lived less than five years. One hundred fourteen persons lived over 10 years and eighteen over 20 years. The longest period of survival was 26 years.

About one-half of the group with onset in the seventh decade (60 to 70 years) lived less than five years. Forty-six lived over 10 years and five over 20 years. The longest survival was 22 years.

Only seven of those with onset in the eighth decade (70 to 80 years) lived more than 10 years thereafter, and the longest survival was 15 years. Those in whom the disease was first recognized after the age of 80 years (ninth decade) were all dead within five years.

The percentage of long-term survivors decreases as the age of onset increases (Table 2). In the group with onset before the age of 40 years about 45% lived more than 10 years and 17% more than 20 years. When the diabetes began between the ages of 40 and 60 years, 31.5% lived more than 10 years and 7% more than 20 years. The diabetic lives of the group with "indefinite" duration probably were nearly all of less than 10 years' duration.

It is clear that diabetes shortens life expectancy to a notable degree. A majority of those who died of diabetic acidosis or infections would have lived longer under proper management, and deaths from these complications are now much less frequent. But diabetics are still careless, and many who have been well controlled, neglect treatment, and coma or pyemia develops. There is also a fairly large group whose diabetes is first recognized after the development of gangrene or coronary thrombosis.

*Incidence of Vascular Disease with Respect to the Age at Death* (Table 3).—In Table 3 the frequency of severe vascular disease is shown with respect to the age at death. Only those cases are included in which the vascular lesion was the cause of death or a major contributing factor. If all those with minor vascular lesions were included, the group would be larger; but this is also true of the nondiabetic group with which the diabetics are to be compared. Since nearly all elderly persons have some degree of arteriosclerosis, we can include for comparison only those in which the arteriosclerosis was the major cause of death.

TABLE 3.—*Incidence and Types of Severe Vascular Disease in Diabetics with Respect to Age at Death*

| Age at Death, Yr. | Total Number of Diabetic Patients | Number with Non-vascular Diseases | Number with Vascular Disease | | | | | | | | Percentage with Vascular Diseases |
|---|---|---|---|---|---|---|---|---|---|---|---|
| | | | Coronary Disease | Encephalomalacia | Intracranial Hemorrhage | Myocardial Failure | Gangrene | Arteriosclerotic Aneurysm | Renal Arteriosclerosis | Total | |
| 0-10 | 9 | 9 | 0 | 0 | 0 | 0 | 0 | 0 | 0 | 0 | 0.0 |
| 10-20 | 25 | 25 | 0 | 0 | 0 | 0 | 0 | 0 | 0 | 0 | 0.0 |
| 20-30 | 51 | 41 | 1 | 0 | 0 | 2 | 1 | 0 | 6 | 10 | 20.0 |
| 30-40 | 89 | 67 | 8 | 0 | 0 | 0 | 1 | 0 | 13 | 22 | 25.0 |
| 40-50 | 123 | 71 | 16 | 9 | 3 | 4 | 7 | 0 | 13 | 52 | 42.3 |
| 50-60 | 300 | 159 | 47 | 14 | 9 | 13 | 36 | 2 | 20 | 141 | 47.0 |
| 60-70 | 512 | 221 | 120 | 26 | 12 | 28 | 82 | 0 | 23 | 291 | 56.8 |
| 70-80 | 256 | 151 | 83 | 31 | 3 | 21 | 61 | 1 | 5 | 205 | 57.6 |
| 80-91 | 90 | 45 | 12 | 12 | 1 | 8 | 10 | 0 | 2 | 45 | 50.0 |
| Total | 1,555 | 789 | 287 | 92 | 29 | 76 | 198 | 3 | 82 | 766 | 49.3 |

It appears in Table 3 that there is a convincing relation between the age at death and the presence of lethal vascular disease. No diabetic under the age of 20 years died of vascular disease. In the combined third and fourth decades 22.8% died of vascular lesions, the most frequent of which was renal arteriosclerosis. In the combined fifth and sixth decades 45.6% were vascular deaths, and after the age of 60 years 56.5%. In the order of frequency the vascular lesions were coronary disease, gangrene, encephalomalacia, renal arteriosclerosis, myocardial failure, intracerebral hemorrhage, and atherosclerotic aneurysm.

*Severe Vascular Disease in Nondiabetics* (Table 4).—In Table 4 all the nondiabetic subjects over 1 year of age from the 1940 and 1948 autopsies are recorded. In 1940 there were 2,082 and in 1948 there were 2,337 such autopsies. The percentage of deaths of vascular disease in the older age groups was about 6% less in 1940 than in 1948 because in the earlier period there were more deaths from tuberculosis, lobar pneumonia, bacterial endocarditis, and syphilis. It is believed, however, that this is a satisfactory control group since 70% of the diabetic deaths occurred after 1939. It appears from Table 4 that vascular death is uncommon in nondiabetics before the age of 40 years but that about one-third of the deaths in persons over 60 years of age are due to vascular disease.

A comparison of the frequencies of the different types of vascular disease in nondiabetics and in diabetics is shown in Table 5. Before the age of 20 years there were no vascular deaths in either group. In the third and fourth decades deaths from vascular lesions were four times as frequent in the diabetics, and in the fifth and sixth decades twice as frequent. After the sixth decade vascular deaths are 71% more frequent in the diabetics. The influence of diabetes in promoting atherosclerosis is striking, and is much more pronounced in younger than in older individuals.

TABLE 4.—*Incidence and Types of Fatal Vascular Disease in Nondiabetics with Respect to Age at Death*

| Age at Death, Yr. | Total No. of Nondiabetic Patients | Number with Nonvascular Diseases | Number with Vascular Disease | | | | | | | | Percentage with Vascular Diseases |
|---|---|---|---|---|---|---|---|---|---|---|---|
| | | | Coronary Disease | Encephalomalacia | Intracranial Hemorrhage | Myocardial Failure | Gangrene | Arteriosclerotic Aneurysm | Renal Arteriosclerosis | Total | |
| 1- 10 | 158 | 158 | 0 | 0 | 0 | 0 | 0 | 0 | 0 | 0 | 0.0 |
| 10- 20 | 120 | 120 | 0 | 0 | 0 | 0 | 0 | 0 | 0 | 0 | 0.0 |
| 20- 30 | 236 | 225 | 1 | 2 | 4 | 0 | 0 | 0 | 4 | 11 | 4.7 |
| 30- 40 | 312 | 292 | 5 | 1 | 6 | 2 | 0 | 0 | 6 | 20 | 6.4 |
| 40- 50 | 443 | 362 | 40 | 5 | 17 | 5 | 0 | 1 | 13 | 81 | 18.3 |
| 50- 60 | 891 | 667 | 105 | 29 | 43 | 32 | 0 | 7 | 8 | 224 | 25.1 |
| 60- 70 | 861 | 650 | 154 | 53 | 40 | 49 | 2 | 8 | 5 | 311 | 32.3 |
| 70- 80 | 879 | 569 | 150 | 72 | 30 | 40 | 5 | 7 | 6 | 310 | 35.2 |
| 80-100 | 424 | 286 | 61 | 30 | 4 | 24 | 4 | 2 | 1 | 126 | 29.7 |
| Total | 4,419 | 3,336 | 516 | 192 | 144 | 152 | 11 | 25 | 43 | 1,083 | 24.5 |

TABLE 5.—*A Comparison of the Frequency of Deaths from Vascular Disease in Diabetics and Nondiabetics of Corresponding Age*

| Age at Death, Yr. | | Total No. of Patients | Percentage Dying of Vascular Disease | | | | | | | |
|---|---|---|---|---|---|---|---|---|---|---|
| | | | Coronary Disease | Encephalomalacia | Intracranial Hemorrhage | Myocardial Failure | Gangrene | Aneurysm | Renal Arteriosclerosis | Total |
| 1- 20 | Nondiabetic | 273 | 0.0 | 0.0 | 0.0 | 0.0 | 0.0 | 0.0 | 0.0 | 0.0 |
| | Diabetic | 34 | 0.0 | 0.0 | 0.0 | 0.0 | 0.0 | 0.0 | 0.0 | 0.0 |
| 20- 40 | Nondiabetic | 548 | 1.1 | 0.5 | 1.8 | 0.4 | 0.0 | 0.0 | 1.8 | 5.7 |
| | Diabetic | 140 | 6.4 | 0.0 | 0.0 | 1.4 | 1.4 | 0.0 | 13.6 | 22.8 |
| 40- 60 | Nondiabetic | 1,334 | 10.9 | 2.6 | 4.5 | 2.8 | 0.0 | 0.6 | 1.5 | 22.9 |
| | Diabetic | 423 | 14.9 | 5.4 | 2.6 | 4.0 | 10.2 | 0.5 | 7.8 | 45.6 |
| 60-100 | Nondiabetic | 2,264 | 16.1 | 6.9 | 3.3 | 5.0 | 0.5 | 0.7 | 0.5 | 32.9 |
| | Diabetic | 668 | 22.4 | 7.2 | 1.7 | 5.9 | 16.0 | 0.1 | 3.1 | 56.4 |
| 40-100 | Nondiabetic | 3,598 | 14.2 | 5.3 | 3.7 | 4.2 | 0.3 | 0.7 | 0.9 | 29.2 |
| | Diabetic | 1,091 | 20.3 | 6.7 | 2.0 | 5.4 | 14.2 | 0.2 | 4.5 | 53.1 |

*Coronary Disease.*—Coronary disease refers to atherosclerosis of the coronary arteries and includes coronary insufficiency from narrowing of the vessels as well as coronary thrombosis. The assignment of a given case to this group was sometimes arbitrary since a subject might have gangrene, myocardial failure, or uremia associated with severe coronary disease. When a large coronary artery was thrombosed the case was classified as coronary disease. This inaccuracy does not affect the total incidence of vascular disease but it may alter the percentages in the different groups.

The number of cases in the third and fourth decades is small, but the striking difference suggests that diabetes accelerates coronary disease in young subjects. After the age of 40 years the incidence of coronary deaths is about 40% greater in diabetics. The ratio is 10 in diabetics to 7 in nondiabetics. This means that out of each 10 coronary deaths in diabetics only 3 are directly attributable to the

diabetes. This is equivalent to stating that 70% of the diabetic subjects who died of coronary disease would be expected to die of this lesion even though they were nondiabetic. Diabetes seems to accelerate the development of atherosclerosis of the coronary arteries, with the result that clinical obstruction of the vessels develops earlier in life and more frequently than in nondiabetics.

*Encephalomalacia.*—Encephalomalacia refers to infarction of the brain due to occlusion of an artery. Only those cases in which it was due to atherosclerosis are included in this group. Its incidence is twice as great in the diabetics in the fifth and sixth decades, but after the age of 60 years the differences are not significant. In the entire group over 40 years of age the incidence of encephalomalacia is 25% greater in diabetics. The ratio is about 5 to 4, which means that in only one of each five cases in which encephalomalacia is found in diabetics is it attributable to the diabetic state.

*Intracranial Hemorrhage.*—In this group are included all forms of spontaneous intracranial hemorrhage due to rupture of an atherosclerotic artery. Subdural and subarachnoid hemorrhages were excluded. Diabetes evidently does not accelerate this lesion, since it is more common in nondiabetics. A patient with no previous history of diabetes who is admitted to the hospital because of acute apoplexy is not classified as a diabetic because of the presence of glycosuria.

*Myocardial Failure.*—Myocardial failure refers to cardiac hypertrophy and general venous hyperemia due to hypertension. It is about 30% more frequent in the diabetics. Cardiac hypertrophy is usually present in those who die of chronic renal insufficiency, but they usually do not have myocardial failure. The moderate increase of this lesion is probably an expression of the greater frequency of vascular hypertension in diabetics. It is probable that this group contains many persons with primary hypertension in whom diabetes develops.

*Gangrene.*—Gangrene practically always involves the lower extremities and is due to atherosclerosis. In a previous publication [4] based upon 49,922 autopsies I found that atherosclerotic gangrene of the lower extremities is about 40 times as frequent in diabetics as in nondiabetics. It accounts for over one-half of the excess of vascular lesions in diabetics over nondiabetics. It is the most serious complication of diabetes in persons past middle life.

*Renal Arteriosclerosis.*—Renal arteriosclerosis in diabetics is somewhat different histologically from the corresponding lesion in nondiabetics. There is a thicker band of hyaline in the arterioles, and often there is a specific glomerular lesion called intercapillary glomerulosclerosis. One never sees the lesions characteristic of malignant hypertension. It is much more frequent in diabetics in the third and fourth decades, and is about five times as frequent in diabetics after the age of 40 years.

It appears, therefore, that the increased incidence of vascular deaths in cases of diabetes is due chiefly to a greater frequency of gangrene, coronary disease, and renal arteriosclerosis. The other forms of vascular disease are not impressively increased by diabetes.

*Relation of Vascular Lesions to the Duration of Diabetes and the Age of Onset* (Table 6).—Onset in the First Decade: None of the 13 persons who lived

4. Bell, E. T.: Incidence of Gangrene of the Extremities in Diabetic and in Nondiabetic Persons, Arch. Path. **49**:469 (April) 1950.

less than 10 years showed any notable vascular disease at autopsy. Three who lived 14, 16, and 18 years, respectively, likewise showed no vascular lesions. One died of renal arteriosclerosis after 17 years. Of those who survived more than 20 years, two died of renal arteriosclerosis after 21 years, one of gangrene after 20 years, and one of myocardial failure and hypertension after 23 years of diabetes. Thus, the four who survived more than 20 years all died of vascular lesions.

Second Decade (10 to 20 Years): Twenty-five of the 60 persons in this group lived less than 10 years, and none showed serious vascular disease at autopsy. Of the 20 subjects who survived from 10 to 20 years, 10 died of renal arteriosclerosis, 1 of coronary disease, and 1 of myocardial failure.

Ten persons survived from 20 to 25 years. Four died of renal arteriosclerosis, two of coronary disease, and one of gangrene. Three subjects who lived 20, 20,

TABLE 6.—*Relation of Vascular Lesions to the Duration of Diabetes and the Age of Onset*

| Onset Decade, Yr. | Total No. of Patients | Duration of Diabetes | | | | | | | | | |
|---|---|---|---|---|---|---|---|---|---|---|---|
| | | 0-10 Yr. | | 10-20 Yr. | | 20-30 Yr. | | 30-42 Yr. | | Indefinite | |
| | | Total No. | No. Vasc.* | Total No. | No. Vasc.* | Total No. | No. Vasc.* | Total No. | No. Vasc.* | Total No. | No. Vasc.* |
| 0-10 | 21 | 13 | 0 | 4 | 1 | 4 | 4 | .. | .. | .. | .. |
| 10-20 | 60 | 25 | 0 | 20 | 12 | 10 | 7 | 1 | 1 | 4 | 0 |
| 20-30 | 77 | 38 | 2 | 18 | 11 | 10 | 7 | 1 | 1 | 10 | 2 |
| 30-40 | 97 | 47 | 5 | 28 | 18 | 14 | 8 | 4 | 3 | 4 | 0 |
| 40-50 | 230 | 113 | 52 | 62 | 40 | 22 | 16 | 4 | 3 | 29 | 6 |
| 50-60 | 414 | 234 | 102 | 96 | 60 | 18 | 12 | .. | .. | 66 | 34 |
| 60-70 | 404 | 283 | 162 | 41 | 30 | 5 | 3 | .. | .. | 75 | 44 |
| 70-80 | 209 | 153 | 79 | 7 | 4 | .. | .. | .. | .. | 49 | 24 |
| 80-91 | 47 | 37 | 20 | .. | .. | .. | .. | .. | .. | 10 | 5 |
| | 1,559 | 943 | 422 | 276 | 176 | 83 | 57 | 10 | 8 | 247 | 115 |
| | | | %† | | %† | | %† | | %† | | %† |
| 0-20 | 81 | 38 | 0.0 | 24 | 54.2 | 14 | 78 | 1 | 100 | 4 | 0.0 |
| 20-40 | 174 | 85 | 8.2 | 46 | 63.0 | 24 | 71 | 5 | 80 | 14 | 14 |
| 40-60 | 644 | 347 | 44.4 | 158 | 63.3 | 40 | 70 | 4 | 75 | 95 | 42 |
| 60-91 | 660 | 472 | 55.2 | 48 | 70.8 | 5 | 60 | .. | .. | 134 | 54 |

* Number with vascular lesions.
† Percentage with vascular lesions.

and 25 years, respectively, had no vascular disease at death, although their diabetes was only fairly well controlled. One died of an infection, one of a hypoglycemic reaction, and one of tuberculosis. One man died of coronary disease at the age of 60 years after 42 years of diabetes. The control of his diabetes was fair but not unusually good.

It appears that diabetes beginning before the age of 20 years is prone to terminate in renal arteriosclerosis after 20 years or more of the disease. Root, Sinden, and Zanca,[5] White,[6] Dolger,[7] and Reuting[8] have noted the marked tendency of young diabetics to have vascular lesions after 20 years or more of diabetes.

5. Root, H. F.; Sinden, R. H., and Zanca, R.: Am. J. Digest. Dis. **17**:179, 1950.

6. White, P., cited by Millard and Root.[5]

7. Dolger, H.: Clinical Evaluation of Vascular Damage in Diabetes Mellitus, J. A. M. A. **134**:1289 (Aug. 16) 1947.

8. Reuting, R. E.: Progress Notes on 50 Diabetic Patients Followed 25 or More Years, Arch. Int. Med. **86**:891 (Dec.) 1950.

Third Decade (20 to 30 Years): Only 2 of the 38 subjects who lived less than 10 years showed severe vascular disease. One died of gangrene after six years of diabetes, and one died of coronary thrombosis at the age of 28 years, one month after the diagnosis was made. It is probable in this instance that mild diabetes had been present much longer. Four of the 18 who lived from 10 to 20 years and 5 of the 10 who survived 20 to 30 years died of renal arteriosclerosis. One man died of gangrene at the age of 68 after 40 years of fairly well-controlled diabetes.

One patient died of aortic stenosis after 20 years, one of osteomyelitis after 23 years, and one of perirenal abscess after 25 years. None of the three had any notable atherosclerosis at autopsy.

Fourth Decade (30 to 40 years): Only five of this group died of vascular disease during the first 10 years. Three died of renal arteriosclerosis after five, eight, and nine years, respectively. One died of gangrene after eight years and one of coronary disease after six months.

Seven of those who survived 10 to 20 years and two who survived more than 20 years died of renal arteriosclerosis. Six of those who survived more than 20 years did not die of vascular disease, but three of them had moderate renal arteriosclerosis.

One subject died of pulmonary tuberculosis after 32 years of diabetes and showed no vascular disease at autopsy. One man died of coronary disease at age 79 after 40 years of excellently controlled diabetes.

Onset After the Age of 40 Years: The duration of the diabetes still influences the frequency of vascular disease, as may be seen in the summary in the lower part of Table 6, but the effect of duration is much less pronounced than in the earlier decades. This is presumably due in part to the inclusion of cases in which the vascular disease was not caused by the diabetes but merely associated with it. Since about one-third of nondiabetics in the older age groups die of vascular disease, only the excess above about 30% may be attributed to the diabetes. It is also probable that in the older age groups the diabetes was present in many cases long before it was diagnosed. In many instances the diabetes was first recognized when the patient was admitted because of gangrene or coronary disease.

Among those with onset between 40 and 50 years there were six who survived 20 to 25 years without having vascular disease; and one person died of carcinoma of the sigmoid 30 years after onset of diabetes, without vascular disease.

In summary, it is evident that when diabetes begins before the age of 40 years vascular lesions seldom develop during the first 10 years of the disease, but thereafter the majority of the patients die of vascular lesions. However, vascular disease does not invariably develop after 20 or even after 30 years of diabetes. Twenty-eight of the 93 persons in this series who lived more than 20 years after onset did not have serious vascular disease at necropsy. In these 28 cases the absence of vascular disease cannot be attributed to better control of diabetes.

The data respecting the relation between vascular disease and the duration of the diabetes shown in Table 6 may be criticized because those who survive for long periods enter age groups in which vascular disease is common in nondiabetics. To get around this difficulty Table 7 was constructed to show the effect of the duration of diabetes upon subjects of corresponding age at the time of death.

It appears from Table 7 that only 2 of 83 persons between the ages of 20 and 40 years died of vascular disease whose diabetes had been present less than 10 years; but 28 of 46 (61%) of those with diabetes of more than 10 years' duration died of vascular lesions. The influence of the duration of the disease in young diabetics is striking.

In the group 40 to 60 years of age the influence of the duration of the disease upon vascular lesions is convincing but not so pronounced as in the younger diabetics.

In the group over 60 years of age there is a significant but only moderate increase in vascular lesions after 10 years of diabetes. Presumably in the older groups there are many vascular deaths for which diabetes is not responsible. Over 30% of nondiabetics over 60 years of age die as a result of vascular lesions.

TABLE 7.—*Incidence of Vascular Deaths in Persons of Corresponding Age with Respect to the Duration of the Diabetes*

| Age at Death, Yr. | Total No. of Diabetics | Duration of Diabetes: 0-10 Yr. Total No. | 0-10 Yr. No. Vasc.* | 10-20 Yr. Total No. | 10-20 Yr. No. Vasc.* | 20-30 Yr. Total No. | 20-30 Yr. No. Vasc.* | 30-42 Yr. Total No. | 30-42 Yr. No. Vasc.* | Indefinite Total No. | Indefinite No. Vasc.* |
|---|---|---|---|---|---|---|---|---|---|---|---|
| 0-10 | 9 | 9 | 0 | .. | .. | .. | .. | .. | .. | .. | .. |
| 10-20 | 25 | 25 | 0 | .. | .. | .. | .. | .. | .. | .. | .. |
| 20-30 | 51 | 32 | 1 | 13 | 5 | 3 | 2 | .. | .. | 3 | 1 |
| 30-40 | 80 | 51 | 1 | 21 | 14 | 9 | 6 | .. | .. | 8 | 1 |
| 40-50 | 123 | 78 | 27 | 26 | 18 | 9 | 6 | .. | .. | 10 | 1 |
| 50-60 | 200 | 103 | 38 | 44 | 20 | 13 | 9 | .. | .. | 40 | 15 |
| 60-70 | 312 | 178 | 148 | 105 | 64 | 20 | 15 | 5 | 3 | 104 | 61 |
| 70-80 | 206 | 105 | 113 | 60 | 39 | 24 | 15 | 5 | 4 | 62 | 34 |
| 80-90 | 90 | 60 | 30 | 9 | 5 | 5 | 3 | 0 | 0 | 16 | 7 |
| | | | %† | | %† | | %† | | %† | | %† |
| 20-40 | 140 | 83 | 0.24 | 34 | 56.0 | 12 | 75 | .. | .. | 11 | 18.2 |
| 40-60 | 423 | 281 | 40.9 | 70 | 67.1 | 22 | 68.2 | .. | .. | 52 | 32.0 |
| 60-91 | 958 | 543 | 53.6 | 174 | 62.1 | 49 | 67.3 | 10 | 70.0 | 182 | 56.0 |

* Number dying of vascular diseases.
† Percentage dying of vascular diseases.

*Influence of the Severity of Diabetes upon the Development of Vascular Disease* (Table 8).—A mildly diabetic person is defined as one whose disease was well controlled without insulin or with small amounts of insulin. None of the persons listed as having "mild" diabetes had polyuria, thirst, or weakness, and none had diabetic acidosis at any time except as a complication of the terminal illness. However, the urine was not kept free of sugar.

Patients with moderately severe and severe diabetes are defined as those whose diabetes could not be controlled without liberal use of insulin. The patients whose diabetes was moderately severe were usually fairly well controlled. Most of those whose diabetes was severe had trouble from time to time and were often out of control.

It will be noted that nearly all the diabetics under 40 years of age are classified as having moderately severe or severe diabetes and a large proportion of the older diabetics as having mild diabetes. This is admittedly a rough separation, but it is well known that a fairly large proportion of elderly diabetics have a mild form of the disease and that many may be well controlled by diet without insulin.

Colwell [9] stated that probably about one-half of older individuals with uncomplicated diabetes could be managed by dietary regulations without insulin.

The group under 40 years of age is too small for statistical study, but in the group beyond this age it appears that there is no significant difference in the incidence of vascular disease in those with mild and those with moderately severe or severe diabetes. This means that the danger of a vascular complication is as great in an individual with mild as in one with severe diabetes.

In seven subjects having diabetes associated with hemochromatosis, ranging in age from 51 to 66 years, there were no vascular complications at autopsy. The duration of the diabetes ranged from four months to six years. The absence of vascular lesions might be due to the short duration of the diabetes, or "bronze diabetes" may have a different causation. Lawrence [10] found no vascular complications in 40 cases of diabetes with hemochromatosis.

TABLE 8.—*A Comparison of the Incidence of Vascular Disease in Mild Diabetes with Its Incidence in Moderate and Severe Diabetes*

| Age at Death, Yr. | Total No. of Diabetics | Mild Diabetes | | | Moderately Severe to Severe Diabetes | | |
|---|---|---|---|---|---|---|---|
| | | | Patients with Vasc. Lesions | | | Patients with Vasc. Lesions | |
| | | No. | No. | % | No. | No. | % |
| 0-10 | 9 | 0 | 0 | 0 | 9 | 0 | 0 |
| 10-20 | 25 | 0 | 0 | 0 | 25 | 0 | 0 |
| 20-30 | 51 | 2 | 0 | 0 | 49 | 10 | 20.4 |
| 30-40 | 89 | 4 | 0 | 0 | 85 | 22 | 25.9 |
| 40-50 | 123 | 32 | 16 | 50 | 91 | 36 | 39.5 |
| 50-60 | 300 | 95 | 44 | 46.3 | 205 | 97 | 47.3 |
| 60-70 | 512 | 223 | 130 | 62.3 | 289 | 155 | 53.6 |
| 70-80 | 356 | 185 | 106 | 57.3 | 171 | 99 | 57.9 |
| 80-91 | 90 | 58 | 27 | 46.5 | 32 | 18 | 56.2 |
| 40-60 | 423 | 127 | 60 | 47.4 | 296 | 133 | 44.9 |
| 60-91 | 958 | 466 | 272 | 58.4 | 492 | 272 | 55.3 |

## COMMENT

When the onset of diabetes is before the age of 40 years, the duration of the disease has a striking influence on the development of vascular lesions (Table 6). That this is not due to age is shown in Table 7. Very few patients show severe vascular disease within the first 10 years of diabetes; thereafter atherosclerosis becomes prominent in various organs. The most frequent vascular complication presented by the younger diabetics is renal arteriosclerosis.

In those who die between the ages of 40 and 60 years (Table 7) the influence of the duration of the disease is not so prominent, and after the age of 60 years the duration exerts only a small influence. This feature is partly explained by Table 5, in which it appears that about 33% of nondiabetics over 60 years of age die from some form of vascular disease. It seems unsound to conclude that all the deaths from vascular disease in older diabetics are attributable to the diabetic state. If we subtract the percentage of nondiabetics dying of vascular disease from the

9. Colwell, A. R.: J. Michigan M. Soc. **49**:417, 1950.

10. Lawrence, R. D.: Physician's Bull. **12**:34, 1947; Brit. M. J. **2**:107, 1950.

percentage of diabetics dying of vascular disease (Table 5), we find that less than one-half of the vascular deaths in diabetics over 40 years of age are due directly to diabetes. Theoretically, perfect control of diabetes in persons past middle life could not reduce the mortality from vascular disease below 30%.

In diabetics who die before the age of 40 years, however, the vascular lesions are due to the presence of diabetes.

Vascular lesions that may be attributed almost entirely to the diabetes are intercapillary glomerulosclerosis and gangrene. Coronary disease is greatly accelerated by diabetes. The accelerating effect of diabetes on other forms of arteriosclerosis is quantitatively not impressive.

Does a diabetic invariably have a severe form of atherosclerosis if he lives long enough? When the onset of the disease was before the age of 40 years, about three-fourths of the subjects in my series died of vascular disease if they survived 20 years or more of diabetes. One does not know what would have happened to the remaining one-fourth if they had lived longer. One knows that in some diabetics vascular lesions do not develop after 20 to 30 years of diabetes. There were 28 subjects in my series without serious vascular disease in whom diabetes had been present for more than 20 years. Two of 10 subjects with diabetes of over 30 years' duration had no vascular disease at autopsy. Very few persons survive more than 30 years of diabetes, and there is not much information in the literature on this group. It may be said that young diabetics who survive the complications of diabetes and intercurrent diseases will probably die ultimately of a vascular disease; but one cannot say that this outcome is inevitable.

When diabetes has its onset after the age of 40 years, over one-half of the vascular deaths may not be assigned to the diabetic state. Over 20% of older diabetics die of diseases entirely unrelated to diabetes.

It is well established that the duration of the diabetic state is a major influence in the development of atherosclerosis in the younger diabetics. Are there any other known influences other than duration?

*Severity of the Diabetes.*—In persons under 40 years of age there are so few cases of mild diabetes that no comparative study can be made of mild and severe forms of the disease (Table 8), but in those over 40 years of age there is no significant difference in the incidence of vascular deaths between those with the mild and those with the more severe diabetes. It may be argued that these data have no value, since the duration of the diabetes is not taken into consideration. The known duration of the diabetes in the two groups averages about the same, but there is more danger of underestimating the duration in a case of mild than in a case of severe diabetes, since the mildly diabetic person is usually asymptomatic until some complication develops. The influence of the duration of the diabetes cannot be eliminated entirely. From the practical standpoint, however, it is clear that in elderly diabetics serious vascular lesions develop as frequently in those with mild diabetes, controllable without insulin, as in those with severe diabetes.

*Influence of Treatment upon the Development of Vascular Lesions.*—Everyone is in agreement that diabetes should be well controlled at all times, that the patient should be kept asymptomatic and without notable glycosuria or hyperglycemia; but there are some who believe that a trace of sugar in the urine or a slight hyperglycemia is not harmful. In practice it is usually difficult to maintain normoglycemia and a normal urine at all times. A patient whose diabetes is under continuous

good control runs little risk of coma or infections and is in a good physical state. There can be no doubt that good control greatly prolongs the diabetic's life. In recent years, however, it has become evident that serious vascular disease usually develops in juvenile diabetics after 20 years or more of the disease although the diabetes has been kept under good control. Dolger [7] believes that even the best control will not prevent the development of vascular lesions in juvenile diabetics. Root and associates,[8] Jackson and others,[11] Daeschner, Deisher and Hartmann,[12] and Joslin and others,[13] however, believe that excellent control will delay and possibly prevent the development of vascular complications.

My observations indicate that the absence of vascular disease in the long-term survivors of this series may not be attributed to better control of the diabetes.

## SUMMARY

This report is based upon a study of 1,559 autopsies made on diabetic subjects.

In about 5% of the group diabetes developed before the age of 20 years; in 16%, before the age of 40 years, and in 70%, after the age of 50 years.

There were no deaths from vascular disease before the age of 20 years. Between the ages of 20 and 40 years, about 23% of the deaths were due to vascular disease. About 54% of those over 50 years of age died of vascular lesions.

About 30% of nondiabetics over 40 years of age die of some form of vascular disease. Therefore, only about 24%, and not 54%, of the deaths of the older diabetics should be attributed to the diabetic state.

The types of vascular disease especially accelerated by diabetes are gangrene, renal arteriosclerosis, and coronary disease.

When the onset of the diabetes is before the age of 40 years, the influence of the duration of the disease on the development of vascular lesions is striking. Few persons present vascular lesions during the first 10 years of diabetes; after 20 years or more of the disease, only few escape.

Diabetes of long duration does not invariably terminate in a vascular death.

In the older diabetics there appears to be no relation between the severity of the diabetes and the tendency toward vascular disease.

In this series, the absence of vascular disease in a few long-term survivors may not be attributed to better control of the diabetes.

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12. Daeschner, C. W.; Deisher, R. W., and Hartmann, A. F.: J. Pediat. **38**:8, 1951.
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# VENOUS VALVULITIS

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CERTAIN changes were encountered in the venous valves in instances of acute and healed valvular endocarditis during an investigation of veins in the various age groups.[1] Though the venous valves have been studied in cases of phlebosclerosis and endophlebitis, systematic studies of these valves in instances of the various types of endocarditis have not been made. The question arose whether or not, in cases of endocarditis, changes occur in the venous valves and, if so, whether they may resemble those seen in the heart valves, especially the aortic and pulmonic valves. Because of the paucity of studies dealing with the valves of the veins in general, the normal venous valve was first studied and will be briefly described.

## ANATOMY AND HISTOLOGY OF THE VENOUS VALVE OF THE FEMORAL VEIN

The femoral venous valve was studied because it resembles grossly the aortic valve. The valve chosen was that between Poupart's ligament and the entrance of the vena profunda. This was regularly encountered in over 200 cases examined, the subjects ranging in age from 2 to 90.

To ascertain the normal histologic appearance of the valve of the femoral vein, serial sections were cut of seven valves. The veins were opened *in situ*, the valves located, and small plugs of cotton soaked in 10% solution of formalin were carefully placed in the sinuses of the valve. The valve, with the adjacent proximal and distal portions of the vein wall, was then removed, allowed to harden in formalin for about a week, embedded in paraffin, and sectioned. Every third section was stained with hematoxylin and eosin, with orcein for elastic fibers, and according to the Van Gieson method for connective tissues, respectively. The ages of these seven patients were 7, 28, 36, 39, 41, 42, and 47. None showed any evidence of increased venous pressure, back pressure, or right heart failure.

The femoral vein valve consists of two cusps. Within the region of the valve the vein is somewhat dilated and its cross section is rather elliptical or oblong. The valve has a convex-shaped base, which is attached to the intima of the vein. The cusps

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This investigation was aided by United States Public Health Service Grant No. H-37 (C-2).

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The Department of Pathology of Michael Reese Hospital is in part supported by the Michael Reese Research Foundation.

1. Saphir, O., and Lev, M.: To be published.

spring from the wall of the vein very close to each other, their free margins diverging from there to become attached again at the opposite region of the wall of the vein. The space between the attachments of the cusps is called the commissure. The commissure itself is slightly raised because of a thickening of the vein wall in that area. Edwards and Edwards [2] named this projection "commissural mound." This mound is much more clearly distinguishable on microscopic section and actually appears as a pedestal from which the cusps seem to arise. The cusps are thicker at their bases, where they join the wall of the vein. This thickened attachment of the cusp framework is called "agger" (Franklin [3]) or "libus" or "tuberculum" as translated from the original German *Wulst* or *Klappenwulst*. The continuations of the free border of the cusp where it meets the vein wall are named the cornua (Franklin [3]) (Fig. 1).

Microscopically, the cusp consists of lining endothelial cells, a lacework of collagen, elastic lamellae, and a very few smooth muscle fibers close to the attachment

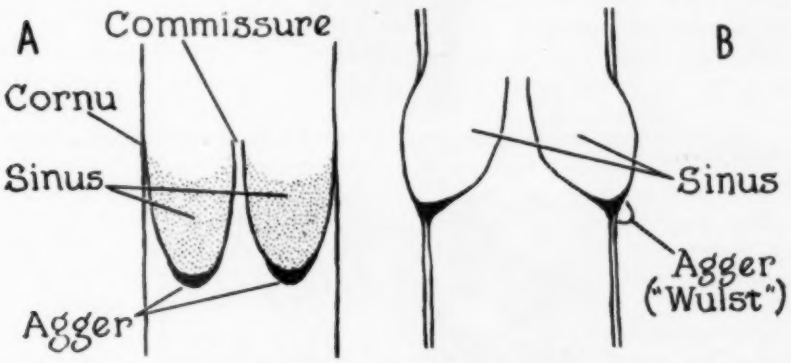


Fig. 1.—Schematic drawings of two views of the femoral vein valve giving the pertinent nomenclature.

of the cusp to the vein wall. For didactic purposes and for better understanding of pathologic changes, the cusp may be arbitrarily divided into two zones, a luminalis and a parietalis (Fig. 2). The luminalis, that part of the cusp close to the lumen of the vein and facing the circulating blood stream, is lined by one layer of endothelial cells, which are elongated in the axis of the vessel. Beneath this layer of endothelial cells is a small amount of connective tissue which is especially noticeable in childhood (Fig. 3). Immediately beneath it is a moderately thick, slightly wavy elastic lamella, the continuation of the internal elastic lamella of the intima of the vein. It extends to the commissure and becomes continuous with that of the luminalis of the adjacent cusp. Occasionally minute elastic fibers branch off into the adjacent collagen tissue of the parietalis. However, this is not the rule, though probably not pathologic.

2. Edwards, J. E., and Edwards, E. A.: The Saphenous Valves in Varicose Veins, Am. Heart J. **19**:338-351, 1940.

3. Franklin, K. J.: Valves in Veins, Proc. Roy. Soc. Med. **21**:1-38, 1927; A Monograph on Veins, Springfield, Ill., Charles C Thomas, Publisher, 1937.

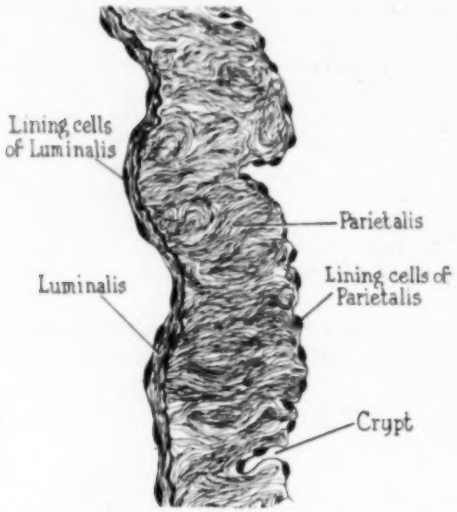


Fig. 2.—Schematic drawing of the microscopic appearance of the valve cusp giving the pertinent nomenclature.

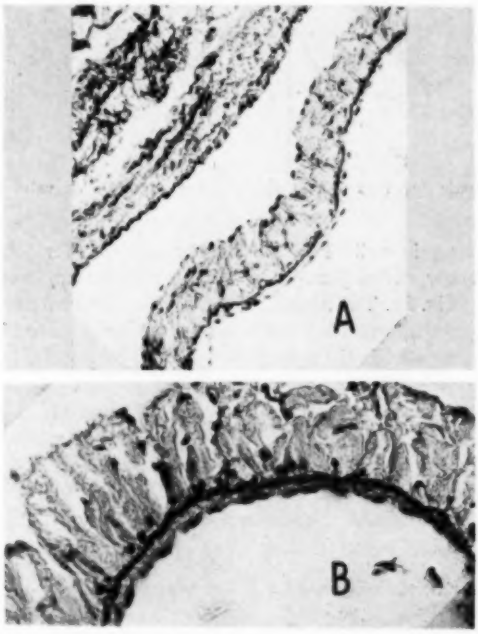


Fig. 3.—*A*, normal cusp of the vein valve. Note the lining endothelium and the elastic membrane of the luminalis. Orcein preparation; × 110. *B*, similar to *A*. Note the crypts. × 280.

The parietalis, that part of the cusp facing the vein wall of the sinus, is lined by one layer of endothelial cells, which are elongated transversely. Whereas the surface of the luminalis appears as a more or less straight line, because of the elastic layer, that of the parietalis on low magnification appears irregular because of minute sinuses or crypts or crevices lined by endothelium, and because of the absence of an elastica (Fig. 3*A*). These crypts, which may be broad or shallow, are not found at regular intervals but are sometimes present only here and there and have to be looked for. In the older age group they seem to be more pronounced. Sometimes they are narrow and slit-like. Their bases occasionally reach the elastic lamella of the luminalis. The remainder of the parietalis in general consists of loosely arranged collagen fibers, which appear the more lace-like the farther away they are from the base and the attachments to the vein wall. A rare connective tissue cell is occasionally present. At the base of the valve the parietalis contains also a few muscle fibers, which extend from the longitudinal muscle layer of the vein wall. These are present in only about the lower fifth of the cusp. There are no blood vessels in the cusps.

The luminalis and the parietalis join or fuse at the distal end of the cusp, which is thinner than the rest. The elastic lamella there is also thinner than elsewhere and sometimes barely perceptible. The lacy collagen is correspondingly thin, and the lining endothelial cells of the luminalis and the parietalis are in close approximation.

In comparing the venous valve cusp with the aortic valve cusp, the following differences are noted: The parietalis of the venous valve does not have an elastica as does the arterialis of the aortic cusp. The latter has a delicate elastic layer, which, however, becomes thinned out and disappears about one-third the way up the valve (Gross and Kugel [4]). The free margin of the venous valve cusp shows neither an aggregation of collagen nor multiplications of elastic lamellae nor anything comparable to the noduli arantii found in the aortic cusp.

The pedestal of the valve or the commissural mound consists of lining endothelial cells, beneath which there is a layer of thick elastic lamellae. There is also a considerable amount of smooth muscle fibers which form the body of the pedestal. These fibers are exaggerations of the internal longitudinal muscle layers of the vein. They are richly interwoven with collagen fibers.

The venous wall of the valve sinus is thinner than the adjacent portions. Between the muscle bundles there are a number of thin elastic lamellae and a homogeneous cement ground substance. At the distal end of the sinus, the muscle fibers join to form the agger of the valve. Franklin [3] maintained that the circular musculature of the vein wall corresponding to the sinus decreases or completely disappears. While it is usually accepted that the media of the vein in the region of the valve practically has only longitudinal muscles, Benninghoff [5] has doubted this. From our studies and those of Benninghoff it seems that there is a peculiar vortex-like arrangement of muscle fibers, connective tissue fibers, and elastic lamellae. This arrangement seems to protect the wall of the sinus better than simple muscle layers. The adventitial fat tissue is increased in this area.

4. Gross, L., and Kugel, M. A.: Topographic Anatomy and Histology of the Valves in the Human Heart, Am. J. Path. **7**:445-474, 1931.

5. Benninghoff, A.: Blutgefässe und Herz, in Handbuch der mikroskopischen Anatomie des Menschen, edited by W. von Möllendorff, Springer-Verlag, 1930, Pt. 1, pp. 149-150.

CHANGES IN THE FEMORAL VENOUS VALVE IN ENDOCARDITIS

In 25 instances of endocarditis, the femoral venous valve between Poupart's ligament and the entry of the femoral vena profunda, or deep vein, were examined, both grossly and histologically. For microscopic examination the entire valve with adjacent portions of the vein wall was removed in toto and allowed to harden in 10%

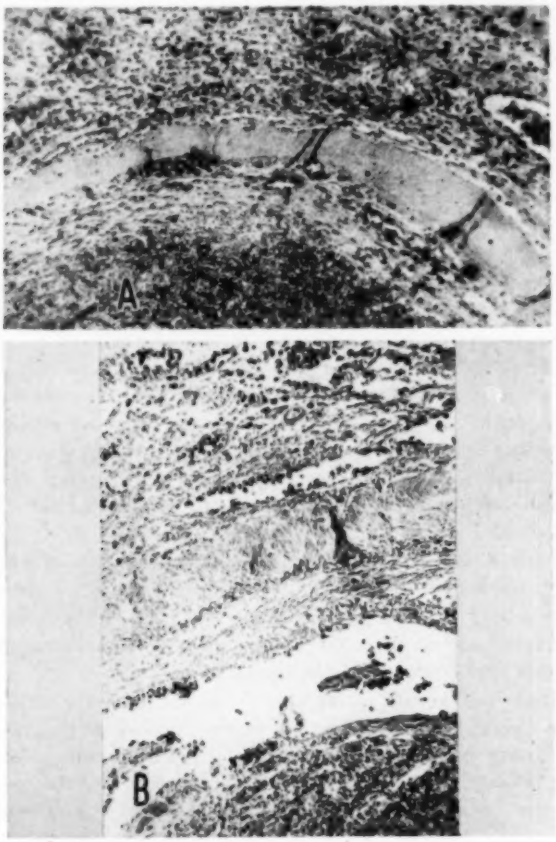


Fig. 4 (Case 1).—*A* shows the ghost-like appearance of the femoral vein cusp. Hematoxylin-eosin preparation: × 90. *B* reveals the indistinguished outlines of the cusp and a barely perceptible elastica of the luminalis. Orcein preparation: × 130.

solution of formalin. Several blocks were cut from each valve either longitudinally or horizontally and embedded in paraffin. In some instances serial sections were cut from the entire valve and in others a various number of sections from each block. Alternate sections were stained with hematoxylin and eosin, orcein, or according to the Van Gieson method. Changes in the femoral venous valve were found in five cases. The details of these cases are here briefly presented:

CASE 1.—The main pertinent anatomic diagnosis was acute bacterial endocarditis superimposed on old endocarditis of the aortic, mitral, and tricuspid valves. The blood culture contained Staphylococcus aureus. The patient was a woman 41 years old.

The right femoral vein, about half way between Poupart's ligament and the opening of the deep vein, contained a yellowish-red mural thrombus not occluding the lumen. On attempting to determine the degree of the mural attachment of the thrombus, a small amount of thin whitish material was noted and first interpreted as fibrin. However, on closer examination it was found to be a membrane-like structure with rough surfaces and definitely not elastic. It was soon realized that this structure represented part of the cusp of the valve of the vein. The vein wall and the thrombus with parts of the cusps were dissected off and hardened in formalin.

Microscopic examination disclosed a variety of changes within the valve. In some portions the cusp was completely necrotic, presenting no cellular elements and appearing as a mere ghost-like structure (Fig. 4*A*). Only mere outlines of the elastica of the luminalis were discernible here and there (Fig. 4*B*). This portion of the cusp seemed embedded in a thrombus which, adjacent to the parietalis, contained many endothelial leukocytes, polymorphonuclear leukocytes, red blood corpuscles, and a number of capillaries. Immediately adjacent to the luminalis there were foci of polymorphonuclear leukocytes with a thin layer of newly formed connective tissue, close to which was a recent thrombus. In this area polymorphonuclear leuko-

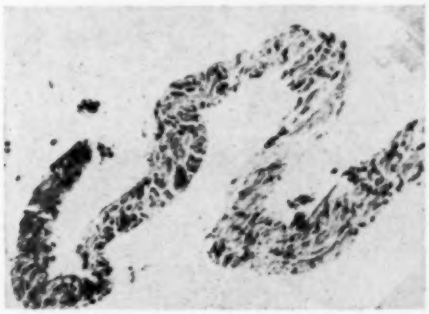

Fig. 5 (Case 1).—Note the polymorphonuclear leukocytes throughout the cusp. Iron-hematoxylin-eosin preparation; × 120.

cytes were found adjacent to the cusp away from the wall of the vein. The remainder of the thrombus consisted of fibrin and red blood corpuscles. In other sections some details of the cusps were recognizable, and a distinction between luminalis and parietalis was still apparent because of the preservation of the elastica of the luminalis. The latter was thicker than normal and seemingly straight. The lining endothelium was absent. Throughout other portions of the cusps were many polymorphonuclear leukocytes (Fig. 5). The Van Gieson-stained sections showed the lace-like collagen of the parietalis replaced by young connective tissue, which was rich in nuclei and which contained a number of young blood vessels. In other fields, small fragments of a cusp, recognizable only as elastic lamellae bordering a necrotic parietalis, were encountered adjacent to a thrombus. In sections taken from the region of the agger of the valve well preserved portions of cusp were demonstrable. However, the distal portions of the cusps were infiltrated with polymorphonuclear leukocytes, lymphocytes, and endothelial leukocytes. Stains for the presence of bacteria could not be employed.

The intima of the vein forming the wall of the sinus contained large numbers of polymorphonuclear leukocytes. The lining endothelium was absent and the subintimal region contained fibrinoid material. The muscle fibers of the media were spread by an edema-like substance, and a few polymorphonuclear leukocytes were also present there. Adjacent to the intima were masses of fibrin and red blood corpuscles but only a few polymorphonuclear leukocytes.

The valve of the left femoral vein on histologic examination proved to be normal.

CASE 2.—The main pertinent gross anatomic diagnosis was abacterial thrombotic endocarditis of the mitral valve superimposed on old endocarditis, thrombosis of the left femoral vein, and pulmonary embolus. The patient was a 28-year-old woman.

The left femoral vein contained a recent red thrombus. The valve was easily identified. The thrombus was attached to the intima just beneath the valve and extended into the opening of the valve and above it. Grossly the valve itself appeared normal. The right femoral vein and its valve showed no gross changes.

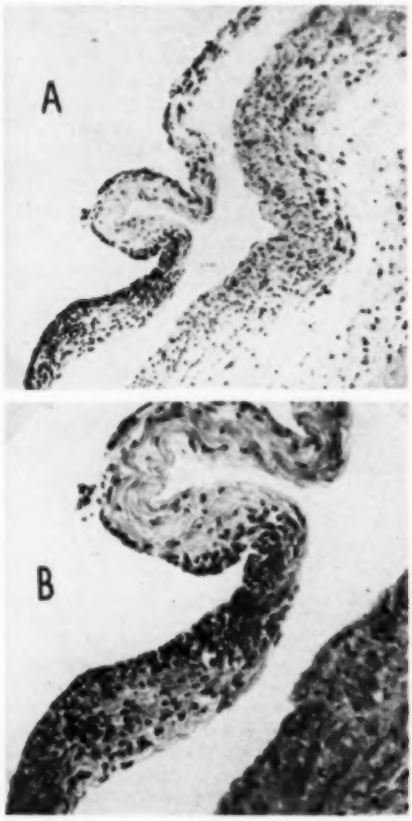


Fig. 6 (Case 2).—*A* shows the masses of polymorphonuclear leukocytes in the cusp. Iron-hematoxylin-eosin preparation; × 90. *B*, similar to *A*; × 160.

Microscopically, in the region of the base of the sinus of the left valve a number of polymorphonuclear leukocytes were noted in the subintimal layer of the wall of the vein, extending from there into the adjacent parietalis of the cusp. In a number of sections the lining cells of the parietalis were well preserved. However, those of the luminalis were focally absent and a small amount of an edema-like material covered the outer aspect of the elastica. In a few sections many inflammatory cells extended through the entire cusp, principally through the parietalis, and were seen in clumps at the distal end of the cusp (Fig. 6). Most of the cells were

polymorphonuclear leukocytes (Fig. 7). In some fields small or large circumscribed accumulations of these cells, intermingled with red blood corpuscles, were attached to the luminalis (Fig. 7*B*). These accumulations resembled vegetations. Sometimes minute fibrinoid-like masses were located between the luminalis and these structures (Fig. 7*C*). The normal lace-like arrangement of the collagen fibers of the parietalis was replaced by much denser fibers which contained a few fibroblasts (Fig. 7*B*). The elastic lamella of the luminalis was definitely thicker than normal (Fig. 7*B*), and here and there multiplication of elastic lamellae was noted. Thin elastic

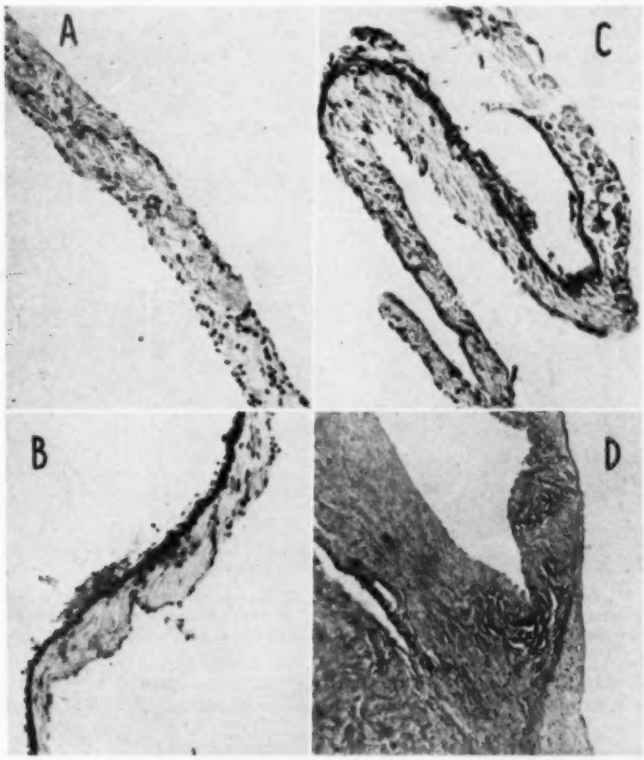


Fig. 7 (Case 2).—*A* shows a few polymorphonuclear leukocytes extending throughout the cusp. Hematoxylin-eosin preparation; × 100. *B* shows polymorphonuclear leukocytes intermingled with red blood corpuscles attached to the luminalis. Orcein preparation; × 100. *C* reveals fibrin attached to the luminalis and a few polymorphonuclear leukocytes throughout the cusp. Orcein preparation; × 160. *D* illustrates the intimal thickening that occurred just beneath the origin of the cusp. Iron-hematoxylin-eosin preparation; × 90.

fibers were also found in the parietalis, particularly at the base of some of the crypts. There were no blood vessels in the cusp.

The wall of the vein showed microscopic changes. Between attachments of the cusps within the commissures there were splitting and multiplications of the subintimal elastic lamellae. The normal configuration of the commissural mound, however, was well preserved. The intimal lining cells of the venous wall of the sinus were absent. The intima was edematous and infiltrated with polymorphonuclear leukocytes.

Microscopic changes were also present in the right femoral vein valve. The elastic lamella of the luminalis was thick and coarse. There were thin elastic fibers at the base of the crypts of the parietalis. An excess of collagen was also found in the latter at the base of the cusp in the region of the agger. The base of the valve close to the vein wall was thickened and contained minute slit-like spaces, obviously closed-off pits, though no red blood corpuscles were found in them. Between the mural attachments of the cusps, particularly between the diverging portions of the cusps, there was a multiplication of elastic fibers of the subintimal region of the vein. Beneath the cusp the intima of the vein disclosed conspicuous proliferation of subintimal connective tissue with a few elastic fibers (Fig. 7*D*). There was also a moderate increase in the inner musculature, but the proliferation of connective tissue and the splitting of the elastic lamella projecting into the lumen of the vein predominated in the histologic picture. Because of this it often seems as if the base of the sinus of the valve were broader than normal, seemingly "sitting" upon the thickened intima of the vein. This process in the intima of the vein is identical with that described by us previously as "endophlebohypertrophy."

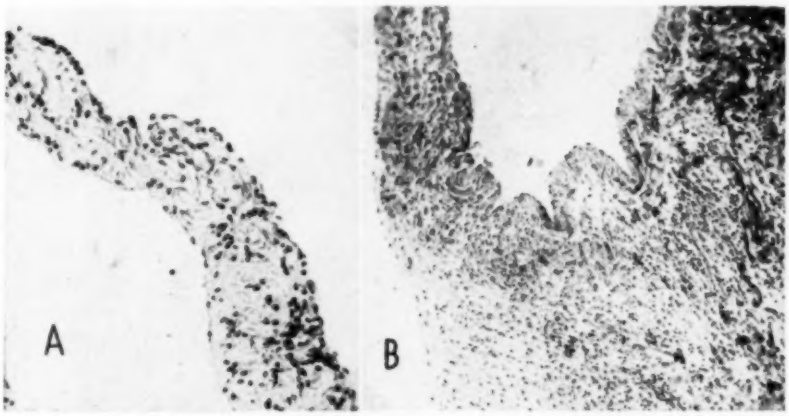


Fig. 8 (Case 3).—*A* reveals infiltration of polymorphonuclear leukocytes at the base of the cusp. Iron-hematoxylin-eosin preparation; × 120. *B* shows polymorphonuclear leukocytes in the vein wall and at the base of the cusp. Hematoxylin-eosin preparation; × 70.

Case 3.—The main anatomic diagnosis was acute rheumatic pancarditis with involvement of the aortic and mitral valves, and old endocarditis of the mitral valve. The patient was a 6-year-old boy.

Both femoral vein valves were grossly normal. On microscopic examination of the left valve there was a moderate infiltration of polymorphonuclear leukocytes and lymphocytes in the subintimal layer of the vein close to the base (agger) of the valve (Fig. 8*A*). The intimal lining cells were preserved. Between the inflammatory cells foci of fibrinoid degeneration were in evidence. Many similar inflammatory cells were present in the region of the commissural mound (Fig. 8*B*). Polymorphonuclear leukocytes and a few lymphocytes, in foci, were also found in the parietalis. In these regions the lining endothelial cells were absent. However the structure of the cusp was well preserved.

No changes were noted in the cusps of the valve of the right femoral vein. The venous wall of the sinus of the valve showed changes similar to those encountered on the left side.

Case 4.—The main pertinent anatomic diagnosis was acute superimposed on chronic rheumatic endocarditis involving the mitral, aortic, and tricuspid valves. The patient was a 29-year-old woman.

Fig. 9 (Case 4).—Note the thickening of the cusp and a few polymorphonuclear leukocytes. Hematoxylin-eosin preparation; × 100.

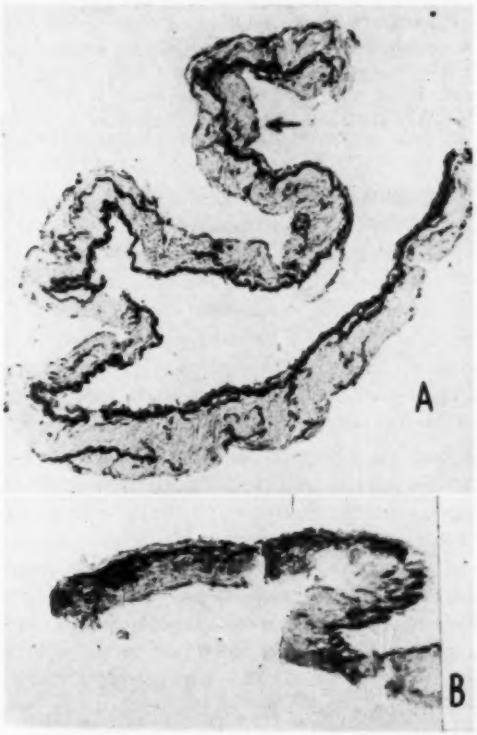


Fig. 10. (Case 5).—*A* shows excess of elastic tissue and a small area of newly formed connective tissue superimposed upon the elastica (pointer). Orcein preparation; × 140. *B* shows dense elastic lamellae at the end of the cusp. Orcein preparation; × 90.

There were no gross changes in either femoral vein valve. Only the left valve was examined by means of serial sections. On microscopic examination the normal structure in general was everywhere preserved. There were a number of polymorphonuclear leukocytes in the midportion of the cusp (Fig. 9). More or less confined to the lateral portions of the cusps adjacent to the commissures, there was an increase in the connective tissue of the parietalis, and the crypts seemed more pronounced. The fibers appeared thicker than normal, and a moderate number of fibroblasts were noted occasionally. Also the base of the cusp showed thick collagen fibers. There was a multiplication of the lining cells of the luminalis in certain foci.

CASE 5.—The main pertinent diagnosis was old endocarditis of the mitral valve with stenosis of its orifice. The patient was a woman 63 years old.

There were no gross changes in the femoral vein valve. On microscopic examination it was noted that the commissural mound was depressed and actually below the level of the adjacent wall of the vein. This region disclosed absence of muscle fibers and masses of thick elastic lamellae but no inflammatory cells. Endophlebohypertrophy as just described was present immediately distal to the valve. The normal structure of the cusps in general was well preserved. However, there was a reduplication of elastic lamellae of the luminalis. Also newly formed elastic fibers were present throughout the parietalis (Fig. 10*A*). An excess of coarse connective tissue was noted in the parietalis, particularly close to its free margin and also in the region of the agger. Crypts were only occasionally seen. A small circumscribed area of newly formed connective tissue was located in the luminalis, between the lining cells and the elastica (Fig. 10*A*, see pointer). The free margins of the cusps disclosed rather dense connective tissue and whirls of elastic lamella, projecting knob-like over the region where the parietalis and the luminalis fuse (Fig. 10*B*). The internal elastic lamella of the vein in the region of the mound was thicker than normal and notably "curled." In a few sections a small, apparently old thrombus was attached to the free end of the cusp. In other fields the sinus was distinctly dilated and a small recent thrombus was located at the base of the sinus. There were no blood vessels in the cusps.

## COMMENT

In 5 cases of endocarditis out of a series of 25, changes were found in the valve of the femoral vein. These five cases comprised one of acute bacterial (Staph. aureus) endocarditis superimposed on old endocarditis, one of a bacterial thrombotic endocarditis superimposed on old endocarditis, two of recent and old rheumatic endocarditis, and one of old endocarditis. In the first case there was complete destruction of the valve cusps with thrombosis; in the second there were a true recent valvulitis, older changes of the valve, and also a recent thrombus; in the third and fourth there were also acute inflammatory lesions, combined with older changes in Case 4. In the last case only old changes were noted.

Regarding the first case, a question immediately arises as to whether the primary lesion was a destructive valvulitis, with secondary thrombosis, or whether an infected thrombus was primary and the destruction of the valve cusps secondary. While it is probably impossible on morphologic grounds to answer this question, the following possibilities may be considered. There was no primary disease of the vein, no evidence of phlebosclerosis. The thrombosis was found only in the valvular region. A primary valvulitis can easily explain secondary thrombosis. If the primary lesion were a thrombus, one would expect the oldest portion of the thrombus to be within the sinus adjacent to the parietalis. This was not so; the oldest portion of the thrombus, as indicated by a thin layer of connective tissue, was attached to the luminalis and the organizing thrombus to the parietalis. Besides, if the primary

lesion were thrombosis, one may assume, as Edwards and Edwards [6] stated, that the valve was destroyed by the organization and recanalization and that any remaining portions were being incorporated in the wall of the vein. Though newly formed blood vessels were present in the cusps, they signify rather end-results of older valvulitis than constituents of organized thrombi incorporated into the cusp. Besides, there were obviously older changes within the cusps, as indicated by the coarse connective tissue replacement of the lace-like parietalis and the presence of young blood vessels slightly farther away from the thrombus. Also the necrosis of the cusps seemed so extensive and severe in some regions that it easily could have caused the formation of the thrombus.

In the second case the evidence favors the interpretation that we are dealing with an older valvulitis and a more recent thrombus. Thus foci of polymorphonuclear leukocytes were often seen in those sections where there was no evidence of thrombus. As a matter of fact, the thrombus probably took its origin from an area just distal to the valve rather than from the valvular area itself. There were also older changes present in the cusps, possibly end-stages of a previous valvulitis. Interesting here was the rare finding of a circumscribed accumulation of polymorphonuclear leukocytes with fibrin or fibrinoid masses somewhat resembling minute vegetations. These were attached to the luminalis. This is in spite of the fact that the severer changes were usually seen in the parietalis.

The changes just beneath the valve, noted in this case, are interesting. The proliferation, in the direction of the blood stream, of the elastic and muscle fibers in the intima has been noted by us to occur normally with increased age. This process has been termed endophlebohypertrophy.[7] This was considered a normal reaction of the vein to mechanical stress. In our cases here presented, however, the areas of thickening greatly exceeded that which might be expected normally. Thickening in excess in size, it was pointed out, may be termed "increased endophlebohypertrophy." This may be explained on the basis of increased stress and abnormal currents occurring just beneath the valve in instances of thickening of the cusps of the valve. This thickening to some extent interferes with the normal flow within the vein. Thus the localized "increased endophlebohypertrophy" may be compared with the localized endocardial thickening one occasionally encountered just beneath a stenosed aortic valve.

In the third case only a moderate acute valvulitis was found. Whether the small amount of fibrinoid material may be significant in the light of the fact that the heart valve lesions were undoubtedly of recent rheumatic nature, we cannot tell. To the unbiased, the valve lesions were definitely not specific. Old lesions were absent. However in the fourth case, which also showed typical acute rheumatic heart lesions, recent and old lesions were seen in the femoral valve. The latter were characterized by an excess of connective tissue, particularly where the luminalis and the parietalis meet at the distal end of the cusp, especially at its lateral margins close to the commissure.

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7. Lev, M., and Saphir, O.: Endophlebohypertrophy and Phlebosclerosis: I. The Popliteal Vein, A. M. A. Arch. Path. **51**:154-178 (Feb.) 1951.

In the fifth case there were only old valvular lesions which were probably the result of an old inflammation. The depression of the commissural mound is similar to that occurring in instances of varicosity of the saphenous vein, as described by Edwards and Edwards.[2] Also, the endophlebohypertrophy encountered beneath the valve is not directly related to the old inflammatory changes, as pointed out above.

As to the causes of the valve changes in the first instance, it seems obvious that the Staph. aureus bacteremia was responsible for the valvulitis. There are no blood vessels in normal venous valves, and therefore organisms must have been brought to the valve by the passing circulating blood. Early and usually more severe changes are found in the parietalis. The elastica of the luminalis, which even in extensive destruction of the cusp is preserved,[8] may prevent organisms from gaining entrance to the remainder of the valve. Normally there are no elastic lamellae in the parietalis. However the crypts of the parietalis could very well constitute breeding places for bacteria in instances of bacteremia and may perhaps be starting points of venous valvulitis As a matter of fact, it seems remarkable that venous valvulitis is not found more frequently in instances of bacteremia. Yet, valves of veins are usually not examined in routine autopsies, and there is no literature on possible lesions of such valves in cases of bacteremia. It is also of interest that in these three cases where acute changes were found in the cusps older lesions were also present. Perhaps the older inflammation made the valve susceptible to the recent inflammation either by reducing the local tissue immunity or mechanically by deepening or widening the crypts as a result of minute scarring. As stated above, normally the crypts are not particularly pronounced or seen in regular intervals. They are more pronounced in instances of older inflammation, as seen in the last two cases.

In the second and third cases the venous valvulitis bore no resemblance to the heart valvular lesions. Here it is conceivable that the acute lesions were the result of a terminal acute infection. In both there was severe bronchopneumonia.

The fourth and fifth cases disclosed old lesions in the valve. Fibrosis and increase in, and splitting of, elastic lamellae were conspicuous. Such changes are also incident to those occurring in senescence. However, as has been pointed out in that study, there are also other changes in, and adjacent to, the valve which are characteristic of advanced age and which could not be found in the cases with endocarditis. Besides there were also present more fibroblasts, which make an inflammatory origin of these valvular lesions more likely.

From the foregoing it would appear that inflammatory lesions of the venous valves are not rare. They seemingly often heal with a moderate degree of fibrosis and an excess of elastic lamellae. Upon such a lesion a new valvulitis may be superimposed. While the changes in the valves of the veins showed no resemblance to those of the heart valves in cases of endocarditis, one factor seems common to both: An old inflammation of both types of valve predisposes them to an acute involvement in instances of bacteremia.

There are no blood vessels in normal vein valves. It is obvious, therefore, that whatever causes venous valvulitis must have been carried by the circulatory blood.

8. Edwards, E. A., and Edwards, J. E.: The Effect of Thrombophlebitis on the Venous Valve, Surg., Gynec. & Obst. **65**:310-320, 1937.

There is a divergence of opinion as to whether bacteria causing valvular endocarditis are carried to the valve by means of intravalvular blood vessels or by the passing blood stream. From our findings of venous valvulitis it may be surmised that probably also in valvular endocarditis the responsible organisms are deposited directly on the valves by the blood stream.

SUMMARY

The normal microscopic appearance of the vein valve is described, and a study of the femoral venous valve in 25 cases of recent and old endocarditis is presented. In four instances acute inflammatory lesions were noted and in three of these also older changes. In one instance only old lesions, most likely the result of acute inflammation, were found. This indicated that inflammatory changes of venous valves are not rare.

# HEPATIC CHANGES FOLLOWING INTRASPLENIC INJECTION OF FATTY SUBSTANCES

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THERE have been numerous attempts to demonstrate a causal relationship between an increase of fat and an increase of fibrous tissue in hepatic cirrhosis. To date there has been no clear demonstration that these are causally related, beyond the observation that certain fats, such as lard and cod liver oil, enhance the development of experimental fibrosis.[1]

The experiments reported here were initiated to study this problem. Fatty substances were injected into the spleen and the evolution of the hepatic changes was followed over a period of months. With this method it was hoped that an agent capable of causing injury to the hepatic mesenchyma would be delivered to the liver over a period of time sufficiently prolonged to induce fibrosis or cirrhosis.

## MATERIAL AND METHODS

The following substances were injected intrasplenically:

1. Cod liver oil
   - (*a*) Cod liver oil.
   - (*b*) Cod liver oil diluted to 50% in gum acacia as given by Hagerty.[2]
   - (*c*) Cod liver oil "neutralized" with calcium hydroxide.
2. Unsaturated fatty acids
   - (*a*) Oleic acid, diluted to 50% in gum acacia.
   - (*b*) Linoleic acid, diluted to 50% and to 25% in gum acacia.
3. Glycerol and liquid petrolatum
4. Rat tissue emulsions
   - (*a*) Emulsion of rat body fat, diluted to 50% in gum acacia. Three grams of retroperitoneal fat of rats was emulsified with 0.75 to 1.0 gm. of powdered acacia and diluted with 27 cc. of distilled water.[2]
   - (*b*) Emulsion of normal liver of rat. This was prepared as described above.
   - (*c*) Emulsion of fatty liver of rat, prepared as described above. The fatty livers were obtained following a high-fat, low-protein diet administered for a period of 8 to 10 weeks.

From the Department of Pathology of The Hebrew University-Hadassah Medical School and the Hadassah University Hospital.

1. Györgi, P.: Liver Injury, in Research in Medical Science, edited by D. E. Green and W. E. Knox, New York, The Macmillan Company, 1950.

2. Hagerty, C. S.: Experimental Embolic Glomerulonephritis Produced with Human Fat, Fatty Acids and Calcium Soaps, Arch. Path. **25**:24 (Jan.) 1938.

White male rats, weighing 100 to 150 gm., of a single strain, were used in all experiments. The animal was anesthetized with ether, the spleen was exteriorized, and the material to be tested was injected directly into the spleen. Often there was slight spilling of the fatty material into the peritoneal cavity from the site of splenic injection. Only one injection was administered. The amounts ranged from 0.15 to 0.5 ml. in the different animals.

The animals were killed at regular intervals as shown in the accompanying table, by etherization and exsanguination.

A few rats were splenectomized 24 hours after intrasplenic injection of the tested material and later killed as noted in the table.

Pieces of right and left lobes of the liver and of the spleen were put into Baker's calcium-cadmium formalin [3] and either Zenker's acetic acid solution or Tellyesniczky's fluid. Frozen sections were cut from the formalin-fixed blocks at 10 μ and stained with the following stains: Sudan IV; a combination of Laidlaw's silver method and Sudan IV; Nile blue sulfate; toluidine blue. Paraffin sections were stained with hematoxylin and eosin, Heidenhain's azocarmine modification of Mallory's connective tissue stain, and Weigert's hematoxylin-Van Gieson stain.

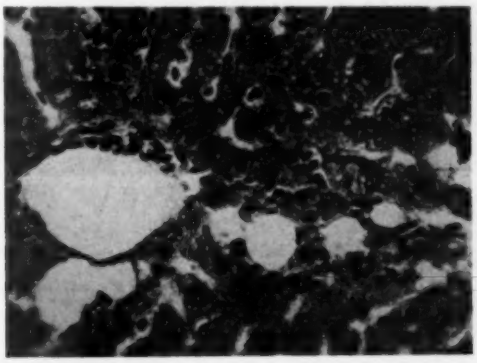

Fig. 1.—Liver five minutes after intrasplenic injection of cod liver oil. Interlobular twigs of the portal vein are distended by oil droplets. × 350. (This and the following pictures were taken from hematoxylin-and-eosin preparations except where otherwise stated.)

## OBSERVATIONS

*Cod Liver Oil.*—Within five minutes after injection, fat droplets were lodged within the portal vascular tree, where they remained for several weeks and disappeared within a month. For the most part the oil lodged in the smaller branches of the portal system, beyond the spaces of Glisson's capsule in vessels which lay between liver lobules (Figs. 1 and 2*A*). Occasionally a small portal branch in Glisson's capsule was occluded by the fatty substance. The greatest number of oil droplets was found in portal vessels beneath the capsule, near the free margin of the lobes. All the changes which occurred took place in relation to the occluding fat droplets. No oil escaped into the sinusoids or the systemic vessels of the liver.

During the first five hours there were accumulations, or small clusters, of polymorphonuclear neutrophiles along the endothelium of the obstructed vessel and around the outside of the vessel. At the end of 24 hours the cellular exudate became a mixture of polymorphonuclear neutrophiles, lymphocytes, and histiocytes. At this

3. Lillie, R. D.: Histopathologic Technic, Philadelphia, The Blakiston Company, 1948.

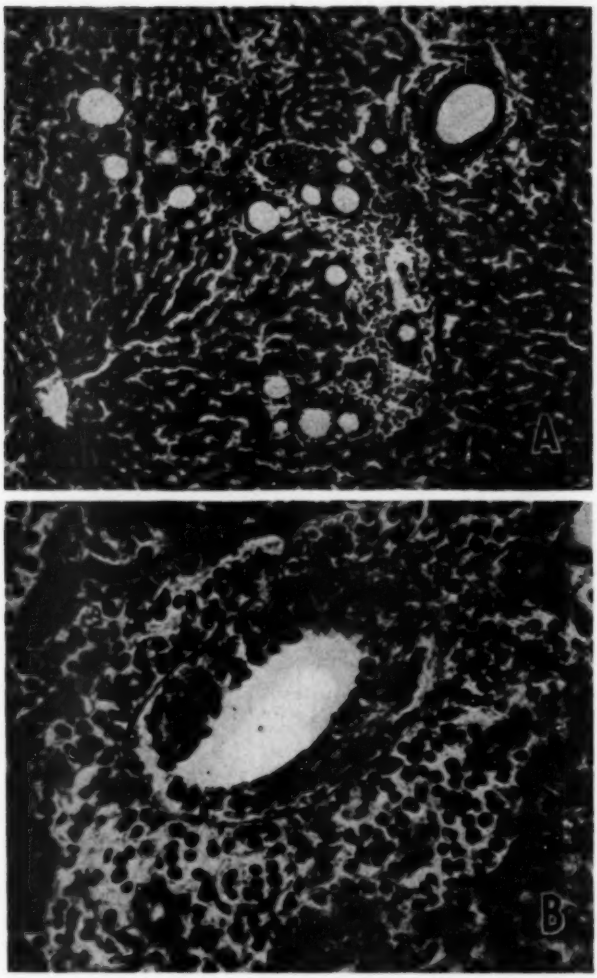


Fig. 2.—*A*, liver 24 hours after injection of cod liver oil. The lobules are delineated by numerous terminal branches of the portal vein, which are distended with oil. The vessels are surrounded by a cellular exudate. × 120.

*B*, liver 24 hours after injection of cod liver oil. The cellular reaction around the vessels is composed of granulocytes in small number, lymphocytes, and histiocytes. The lumen is narrowed by proliferation of endotheloid cells. × 420.

time, too, at the site of the droplet the lumen of the vessel was narrowed by increased layers of flattened cells resembling endothelium (Fig. 2). Liver cords at the periphery of the lobule and in close relation to the occluded vessel showed evidence of coagulation necrosis involving a considerable portion of the lobule. At the borders of the necrotic areas the cytoplasm of the viable hepatic cells was markedly basophilic, and it was here that scattered small clusters of polymorphonuclear leucocytes were seen.

At the end of two days the intravascular space about the oil droplet was almost completely occluded by cells which appeared to be derived from endothelium. Some of these cells contained minute fat droplets in their cytoplasm. In the parenchyma the alterations were transient and moderate in extent. After the necrosis which was seen in the first 24 hours, the dead cells were no longer found at 48 hours, only the reticular structure remaining. Numerous mitoses of liver cells were visible at this time (Fig. 3*A*), and minute fat droplets were seen within the hepatic cells at the periphery of the lobules. Four days after oil injection the hepatic parenchyma was completely without alterations and remained so up to three months of observation.

The intravascular lesion, however, progressed until, one week after injection, numerous granulomas were present at the sites of oil-droplet obstruction (Fig. 3*B*). At this time the liver lobule was well delineated by interlobular inflammation and by interlobular vessels, which appeared increased in number as compared with the number found in control livers. Beneath the capsule the greatest number of granulomas was found, but here no definite pattern was discernible.

In the center of some of the granulomas there was a single large sudanophilic droplet, surrounded by macrophages and one or more foreign-body giant cells (Fig. 4*A*), which often contained engulfed sudanophilic material. The granuloma itself was fairly well demarcated, and at its periphery, outside the vessel and in its wall, were aggregates of lymphocytes, polymorphonuclear neutrophiles, and histiocytic elements. At this time, too, reticulum and collagen preparations showed an obvious increase of fibers around the vessels, merging with the periphery of the granulomas and in some instances reaching to Glisson's spaces (Fig. 4*B*). In the subcapsular region these new strands of connective tissue occasionally surrounded small groups of hepatic cells to form pseudolobules. In areas other than the subcapsular region the fibrous tissue did not extend into the lobule. Rarely a focal granulomatous lesion was found on the capsular surface binding omental tissue to the liver. Elsewhere the capsule remained uninvolved.

At two weeks the granulomas appeared decreased in size and numbers. Lipid-filled macrophages were found at the site of granulomas in the newly formed fibrous tissue and also in Glisson's spaces. The size of the occluding droplet in the center of the granuloma was diminished and an increased number of macrophages with small sudanophilic droplets were visible.

Three weeks after injection few vascular lesions were present. In place of the granulomas one now found discrete scars (Fig. 5*A*) or accumulations of macrophages, many of which contained sudanophilic material in large vacuoles (Fig. 5*B*). The fibrous tissue and the cellular infiltrate about the interlobular vessels were diminished and the hepatic lobules were no longer clearly delineated. Here only sparse collections of lymphocytes remained. An increased number of lipid-filled macrophages were also present in Glisson's spaces.

At four weeks the liver was almost normal. Rarely a few strands of connective tissue, forming pseudolobules, might be found (Fig. 6*A*). Lipid-filled macrophages and lymphocytes were still numerous in Glisson's spaces. In some of the portal

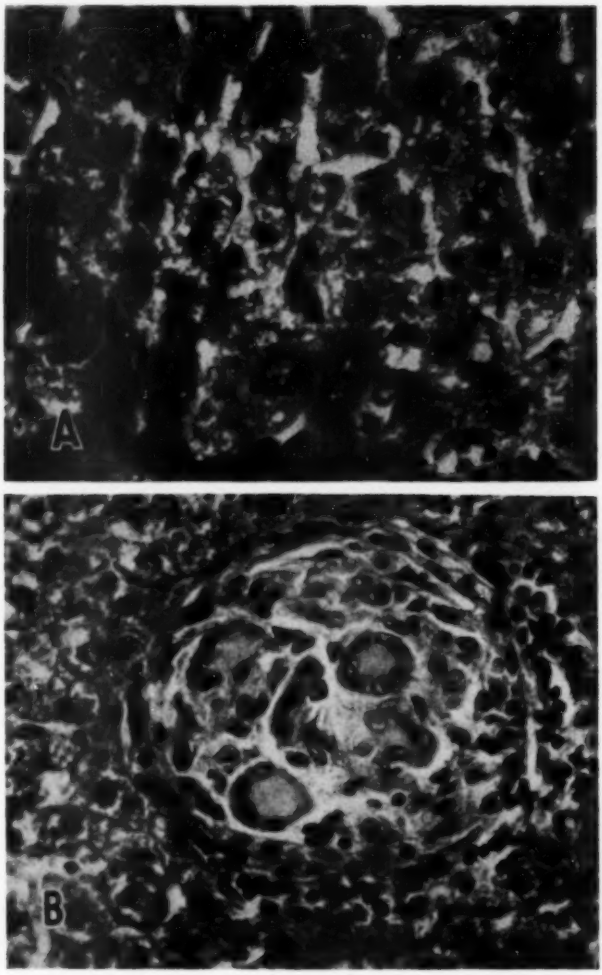


Fig. 3.—*A*, liver 48 hours after injection of cod liver oil, showing mitoses of liver cells. × 550.

*B*, liver seven days after injection of cod liver oil. A granuloma with giant cells occludes a small branch of the portal vein. The stretched vessel wall can be distinctly recognized. × 500.

vessels the lumens were narrowed by organized mural thrombi (Fig. 6*B*). A slight increase in the number of capillaries was discernible in Glisson's spaces.

Beyond four weeks the only evidence of the previous process was the presence of macrophages with large vacuoles in scattered areas corresponding to the sites of earlier granulomas and in Glisson's spaces.

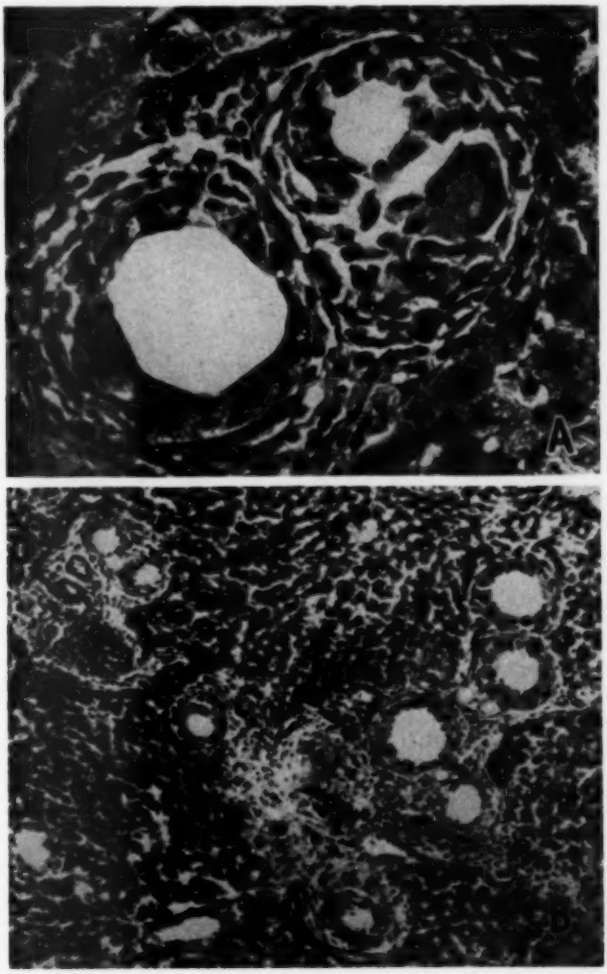


Fig. 4.—*A*, liver seven days after injection of cod liver oil. The intravascular granuloma is composed of endothelioid foam cells and giant cells. The lumen of the vessel is filled by large oil droplets. × 500.

*B*, liver 10 days after injection of cod liver oil. Fibrous strands are seen connecting the involved small vessels with the periportal connective tissue and in several places with sublobular veins. × 45.

The lipid material in the phagocytes, which were first seen three weeks after intrasplenic injection of cod liver oil, was apparently not lost during the process

of embedding, and with the Ziehl-Neelsen technique it stained dark red. In hematoxylin-eosin-stained paraffin preparations many of the phagocytes contained fine reddish-brown granules. The Gömöri modification of the Prussian blue reaction revealed in many of the lipid-filled macrophages dark-blue irregular granules with

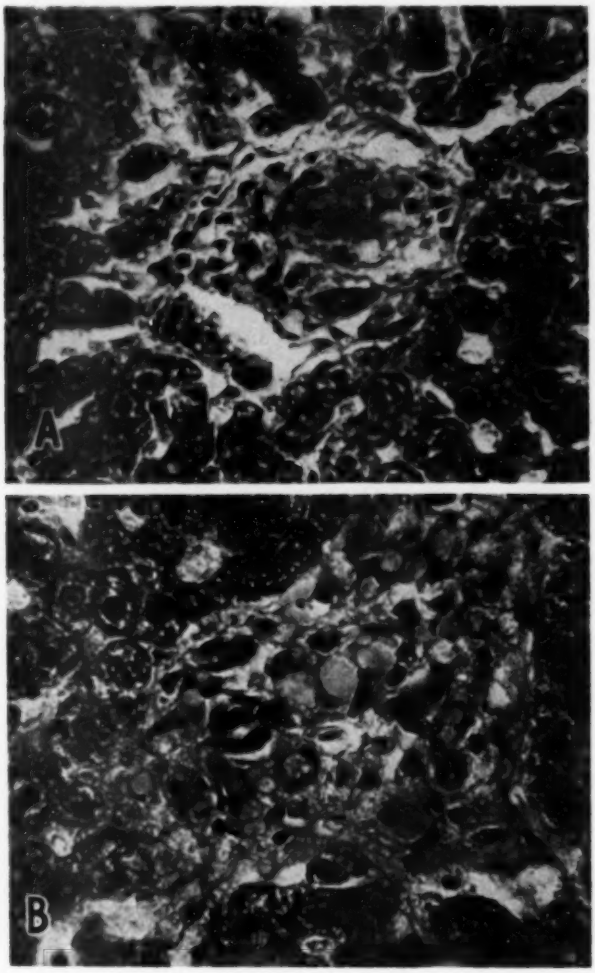


Fig. 5.—*A*, liver three weeks after injection of cod liver oil. A fibrous scar has replaced the granuloma, surrounded by young capillaries. × 430.

*B*, liver three weeks after injection of cod liver oil. There are accumulations of phagocytes at the site of earlier granulomas. The cells contain sudanophilic and ceroid material. × 540.

or without yellow-pigment granules. This was not a constant finding; all cells in the same liver with acid-fast lipid did not show a positive iron reaction, and in some

rats no iron was found despite the presence of numerous lipid-filled cells. Nor was there any correlation between the presence of iron and the age of the process.

In the liver of one animal three weeks after oil injection a single large oil droplet occluded the lumen of a vessel. This droplet was acid-fast with Ziehl-Neelsen stain

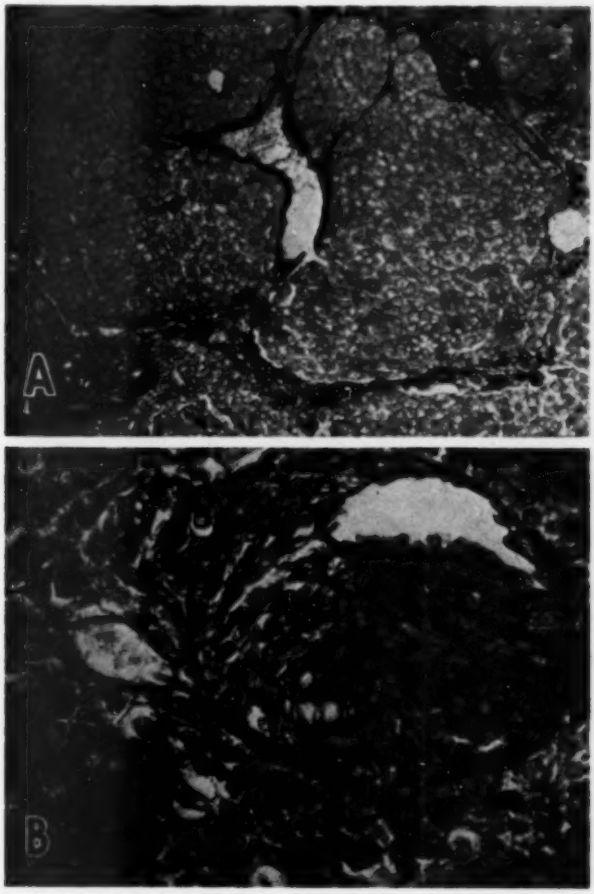


Fig. 6.—*A*, liver four weeks after injection of cod liver oil. Thin strands of connective tissue surround the lobules peripherally, extend to the spaces of Glisson's capsule and also to sublobular veins. Heidenhain's azocarmine modification of Mallory's connective tissue stain. × 100

*B*, liver four weeks after injection of cod liver oil, showing an organized mural thrombosis in a terminal portal ramification. Heidenhain's azocarmine Mallory stain. × 500.

and was not dissolved during the process of embedding in paraffin. Phagocytic cells surrounding the globule contained minute acid-fast droplets.

The above series of events also occurred following the injection of 50% cod liver oil in acacia and following that of "neutralized" cod liver oil.

Five animals were splenectomized 24 hours after the intrasplenic injection of cod liver oil. The same sequence of lesions was visible in this group as described above for the nonsplenectomized group.

*Unsaturated Fatty Acids.*—The injection of 50% oleic acid or of 25 or 50% linoleic acid in acacia produced similar histological responses in the liver, varying only in a few minor details.

Three rats died within 24 hours after injection of 50% oleic acid, and four died after administration of 50% linoleic acid. The remaining animals were killed as indicated in the table.

The livers of animals which died within 24 hours after injection of an unsaturated fatty acid showed extensive necrosis. The necrotic areas were chiefly peripheral but occasionally extended toward the central vein. Sinusoidal dilatation and congestion were conspicuous, and hemorrhages were numerous, particularly into the

*Interval Period and Number of Animals Killed After Intrasplenic Injection of Tested Material*

| | Days | | | | | | | | | Total Number of Animals |
|---|---|---|---|---|---|---|---|---|---|---|
| | 0-½ | 1 | 2 | 4-5 | 7-10 | 14 | 21 | 28 | 60-120 | |
| Cod liver oil | 3 | 2 | 2 | 3 | 11 | 3 | 1 | 1 | 5 | 31 |
| 50% cod liver oil in acacia | 1 | 2 | 2 | 2 | 2 | 2 | 1 | 1 | 0 | 13 |
| "Neutralized" cod liver oil | 0 | 4 | 3 | 2 | 3 | 2 | 1 | 0 | 0 | 15 |
| Oleic acid 50% | 4 | 3 | 3 | 2 | 4 | 1 | 1 | 0 | 0 | 18 |
| Linoleic acid 50% | 2 | 1 | 3 | 0 | 1 | 0 | 0 | 0 | 0 | 7 |
| Linoleic acid 25% | 0 | 3 | 0 | 1 | 3 | 1 | 1 | 1 | 0 | 10 |
| Liquid petrolatum | 0 | 2 | 2 | 1 | 1 | 1 | 0 | 1 | 0 | 8 |
| Glycerol | 0 | 2 | 3 | 2 | 2 | 1 | 0 | 0 | 0 | 10 |
| Emulsion of rat body fat | 1 | 4 | 2 | 2 | 2 | 0 | 0 | 0 | 0 | 11 |
| Emulsion of fatty rat liver | 0 | 2 | 2 | 2 | 2 | 1 | 1 | 0 | 0 | 10 |
| Emulsion of normal rat liver | 0 | 1 | 1 | 1 | 1 | 0 | 1 | 0 | 0 | 5 |
| Cod liver oil; splenectomy 24 hr. later | 0 | 0 | 0 | 0 | 3 | 2 | 0 | 0 | 0 | 5 |
| Total | | | | | | | | | | 143 |

necrotic regions. In the livers of the rats receiving linoleic acid there was a striking polymorphonuclear neutrophile infiltration in and around the necrotic foci as well as a scattering of these cells in the sinusoids. In the livers of the oleic acid series there was no polymorphonuclear neutrophile reaction.

At 48 hours the same extensive necrosis and focal hemorrhage were still visible. In addition, there were numerous mitoses throughout the remaining uninvolved parenchyma. Most of the viable hepatic cells contained small sudanophilic droplets, and in several areas larger fat droplets were lying free in sinusoids. In the oleic acid series the polymorphonuclear neutrophile response was sparse, while it was abundant in the linoleic acid group.

After 4 days the liver parenchyma appeared wholly intact, and the only vestige of injury was the presence of moderately increased numbers of lymphocytes in Glisson's spaces. Occasionally a variable number of lymphoid cells were seen within sinusoids without a particular pattern of distribution.

In all rats that survived a week or more, all the lobes of the liver were fused together, and the capsule was slightly opaque grossly but still glistening. Microscopically, the capsule was markedly thickened, at first by loose connective tissue and young fibroblasts, later by dense, less cellular fibrous tissue (Fig. 7*A*).

In none of the rats of this series was there any evidence of intra- or extra-vascular granulomas or of fibrous tissue increase in the parenchyma.

*Glycerol and Liquid Petrolatum.*—After a single intrasplenic injection of glycerol (0.3 cc.) there were at 24 hours small foci of hepatic cell necrosis near Glisson's

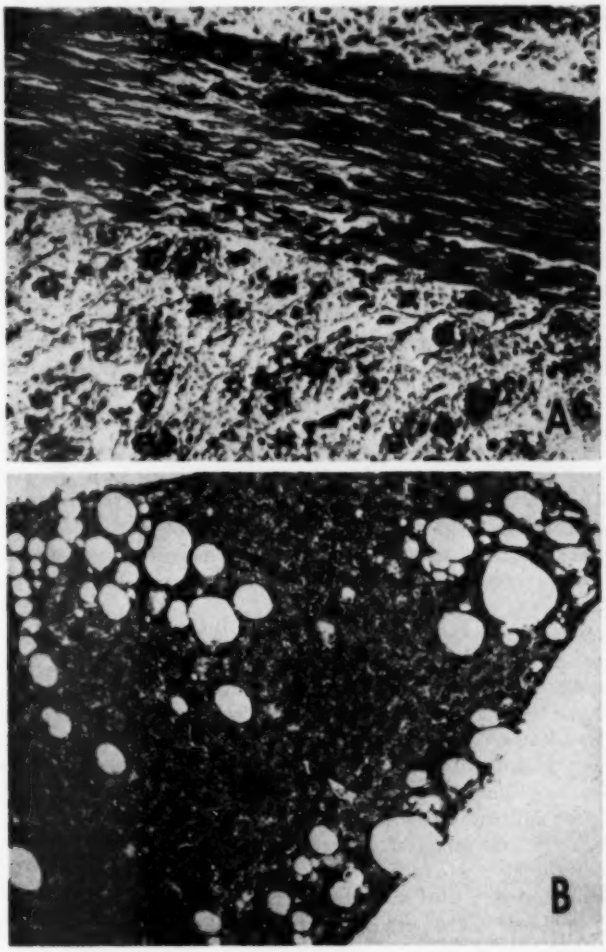


Fig. 7.—*A*, liver seven days after injection of linoleic acid. There is advanced thickening of the capsule by cellular connective tissue. × 450.

*B*, liver seven days after injection of paraffin oil. Vessels are dilated by paraffin oil with no surrounding reaction. × 135.

spaces and a moderate infiltration of polymorphonuclear neutrophiles was found in and around these foci. Twenty-four hours later the necrotic areas were decreased in number and size, the polymorphonuclear neutrophile infiltration was sparse, and

large macrophages appeared peripherally at the damaged sites. No fat droplets were visible either in vessels or in hepatic cells. Beyond two days the liver appeared almost normal, showing only an occasional Glisson's space containing small numbers of lymphocytes. In the two rats studied four days after injection of oil several areas beneath the capsule showed a few fine strands of connective tissue extending from the central vein through the lobule (Fig. 7*B*). Beyond this time there were no alterations.

Liquid petrolatum, in the first 24 hours, caused only dilatation of smaller branches of the portal tree beyond Glisson's sheath and necrosis of a few hepatic cells at the sites of oil obstruction. There were accumulations of lymphoid cells surrounding these dead cells but no reaction around the obstructed vessel. At four days and up to four weeks after administration, the liquid petrolatum droplets were seen in the same sites and still without surrounding reaction (Fig. 7*B*). No observations were made beyond this time.

*Emulsions of Rat Tissues.*—Rat body fat emulsified in acacia caused only minor histological changes. Within the first four days sudanophilic material was occasionally found in the lumina of large portal vessels lying in Glisson's spaces. Contiguous to these obstructed vessels a few hepatic cells were necrotic, and there was a slight infiltration of polymorphonuclear neutrophiles around these cells. Beyond four days the liver appeared intact.

Injections of emulsions of normal rat liver resulted in occasional necrotic foci within the hepatic lobule which disappeared at 48 hours. In Glisson's spaces plasma and lymphoid cells accumulated in small numbers up to one week. Beyond this time the livers were normal.

Injections of emulsions of fatty rat liver were followed by no changes except a variable and slight increase of lymphoid cells in some of the portal spaces.

## COMMENT

The present experiments were undertaken to analyze some of the factors likely to be effective in the production of fibrosis or cirrhosis in the liver. Fat substances were selected for injection because of their known irritating action on connective tissue and also because of their debated role in the pathogenesis of cirrhosis. Several authors have demonstrated a marked proliferation of collagenous tissue in the presence of unsaturated fatty acids and their soaps,[4] and Tompkins[5] observed the development of granulomas in subcutaneous tissues following local administration of phospholipids. Moreover, it was reported that, following hydrolysis of neutral fats in tissues in vivo, the inflammatory reaction was due to the presence of liberated fatty acids. The glycerol fraction was said to be without effect.[4b] Glycerol and liquid petrolatum were used as control materials for the cod liver oil, fatty acids, and tissue emulsions. The purpose was to study the effects of vascular obstruction caused by inert oily substances.

4. (*a*) Hirsch, E. F.: Embolic Pulmonary Lesions Produced in Rabbits by Human Fat Containing Fatty Acids or Soaps, Arch. Path. **21**:765 (June) 1936; (*b*) Relation of the Chemical Composition of Lipids to Characteristic Tissue Lesions, Arch. Path. **31**:516 (April) 1941. (*c*) Hass, G. M.: Tissue Reactions to Natural Oils and Fractions Thereof, Arch. Path. **26**:596 (Nov.) 1938. (*d*) Hagerty.[3] (*e*) Lillie.[3]

5. Tompkins, E. H.: The Reaction of the Subcutaneous Tissues to the Acetone-Insoluble Lipoids from Beef Brains, Bull. Johns Hopkins Hosp. **70**:55, 1942.

This investigation revealed several significant facts. First, the different lipid materials were all effective in producing a greater or lesser degree of hepatic cell necrosis. But only cod liver oil elicited the granulomatous and fibrotic response. In explanation, this may possibly be ascribed to its chemical composition rather than to ischemia caused by oil-plugging of blood vessels. This conclusion seems plausible since no alterations were found in the livers of animals receiving injections of glycerol or liquid petrolatum, other than a transient and slight necrosis. According to Dr. F. A. Vandenheuvel,[6] organic chemist, the Fisheries Research Board of Canada, about 98% of cod liver oil consists of a great variety of simple and mixed fatty acid glycerides, the rest being unsaponifiable matter. About 87% of the fatty acids are unsaturated, having from one to six double bonds. With such a mixture it is not possible to determine in the present experiments what fractions may be responsible for the observed changes.

Second, there was a temporal dissociation between the parenchymal necrosis and the development of vascular granulomas and perivascular fibrosis. The necrosis appeared in 24 hours and was completely absent four days after cod liver oil administration. The granulomas, however, were first seen between two and four days, progressed for a period of 10 more days, and then regressed. Accompanying their development and disappearance was a slight interlobular perivascular fibrosis which increased and then decreased with the granulomatous lesions.

The fibrotic response was apparently reversible. In the livers of all animals allowed to live from 10 to 14 days after administration of cod liver oil there was always an increase of the interlobular perivascular connective tissue. In the livers of all animals allowed to live up to four weeks or later, this was not seen. These observations corroborate the findings of others who have reported a regression in the fibrotic response after cessation of the employment of hepatic poisons.[7]

It has been shown in experimental protracted carbon tetrachloride poisoning of rats that beyond a certain period fibrosis was accompanied by transformation of the hepatic blood supply, and then the lesion became irreversible.[8] In the present experiments the changes developed following a single dose of an irritant, and we cannot at present state whether repeated doses might have resulted in an irreversible type of fibrosis.

At the outset it was expected that a single intrasplenic injection would afford a continuous flow of fatty substances to the liver over a period of several weeks until the depot in the spleen was exhausted. We found, however, the same results in the liver when the spleen was removed 24 hours after intrasplenic injection of cod liver oil. Further, the same hepatic lesions developed in several rats, not reported here, which received repeated intrasplenic injections of the oil. These findings indicated

6. Vandenheuvel, F. A.: Personal communication to the authors.

7. Cameron, G. R., and Karunaratne, W. A. E.: Carbon Tetrachloride Cirrhosis in Relation to Liver Regeneration, J. Path. & Bact. **42**:1, 1936. Steinberg, B., and Martin, R. A.: Absorption of Scar Tissue in Experimental Nodular Cirrhosis of the Liver, Arch. Path. **41**:1 (Jan.) 1946. Morrione, T. G.: Factors Influencing Collagen Content in Experimental Cirrhosis, Am. J. Path. **25**:273, 1949.

8. Ungar, H.: Transformation of the Hepatic Vasculature of Rats Following Protracted Experimental Poisoning with Carbon Tetrachloride, Am. J. Path. **27**:871, 1951.

that the hepatic damage occurred very soon after a single injection of oil and that little or no additional fatty substances were being supplied to the liver after the first one to two days.

Finally, only in the livers of rats given cod liver oil were phagocytes seen containing lipid material which was acid-fast, preserved during the process of embedding, and in some instances showing an iron-positive reaction. The intracellular material was most probably ceroid according to the definitions of Endicott and Lillie [9] and Lee.[10] It was likely derived from one or more of the components of cod liver oil other than oleic and linoleic acids, for injection of the latter unsaturated fatty acids did not result in the appearance of ceroid. Pinkerton [11] made similar observations in his study of cod liver oil pneumonia.

The present investigation supports to some extent the theory that hepatic cirrhosis is the result of selective irritation of the mesenchyma with or without simultaneous parenchymal injury. This idea was suggested years ago by Rössle [12] and more recently by Gillman and Chaikoff.[13] There is, however, an obscure factor which seems to predispose to the development of intrahepatic fibrosis. Oleic and linoleic acids and liquid petrolatum cause a connective tissue increase elsewhere in the body. In our experiments oleic and linoleic acids produced a conspicuous capsular fibrosis of the liver. Yet, in the liver, despite the extensive parenchymal necrosis, there was no fibrous tissue response. Liquid petrolatum likewise produced no reaction within the liver. These observations suggest that the liver cell may be capable of inhibiting fibrous tissue formation, at least for some irritant substances.

## SUMMARY

Cod liver oil injected into the spleen produced parenchymal injury in the liver within 24 hours. This injury disappeared in four days. Intravascular granulomas developed after two days, progressed in size and number up to two weeks, and finally disappeared after four weeks. Interlobular perivascular fibrosis developed within 10 days and regressed after two weeks. Ceroid in macrophages was first seen three weeks after administration of cod liver oil and was present up to three months, the period of observation.

Unsaturated fatty acids (linoleic and oleic), oily substances (glycerol and liquid petrolatum) and emulsions of rat body and liver fat were also injected into the spleen. These agents caused a variable and transient parenchymal injury without mesenchymal response. Ceroid was not found in the liver after the administration of these substances.

9. Endicott, K. M., and Lillie, R. D.: Ceroid, the Pigment of Dietary Cirrhosis of Rats, Am. J. Path. **20**:149, 1951.

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# Books

CURRENT THERAPY 1952: LATEST APPROVED METHODS OF TREATMENT FOR THE PRACTICING PHYSICIAN. By Howard F. Conn, M.D., editor, with 10 consulting editors. Pp. 849. Price $11. W. B. Saunders Company, 218 W. Washington Sq., Philadelphia 5; 7 Grape St., Shaftesbury Ave., London, W.C. 2, 1952.

CELLULAR CHANGES WITH AGE. By Warren Andrew, Ph.D., M.D., professor of anatomy and chairman of department, The George Washington University School of Medicine, Washington, D. C. Pp. 74, with 25 illustrations. Price $2.50. Charles C Thomas, Publisher, 301-327 E. Lawrence Ave., Springfield, Ill., 1952.

LEHRBUCH DER RÖNTGENDIAGNOSTIK. By H. R. Schinz, W. E. Baensch, E. Friedl, and E. Uehlinger. Pp. 514, with 674 illustrations and 30 plates. Price 84 German marks. Georg Thieme, Diemershaldenstrasse 47, (14a), Stuttgart O; Agents for U. S. A.: Grune & Stratton, Inc., 381 Fourth Ave., New York 16, 1951.

UEBER DIE RÖNTGENOLOGISCHEN DARSTELLUNGSMÖGLICHKEITEN DES WEIBLICHEN GENITALAPPARATES MIT HILFE VON JODÖL UND JODSOL. By Doz. Dr. J. Erbslöh. Pp. 74, with 64 illustrations. Price 16.80 German marks. Georg Thieme, Diemershaldenstrasse 47, (14a), Stuttgart O; Agents for U. S. A.: Grune & Stratton, Inc., 381 Fourth Ave., New York 16, 1951.

RHEUMATIC DISEASES BASED ON THE PROCEEDINGS OF THE SEVENTH INTERNATIONAL CONGRESS ON RHEUMATIC DISEASES. Prepared by the Committee on Publications of the American Rheumatism Association; Charles H. Slocumb, M.D., chairman. Pp. 449, with 126 figures. Price $12. W. B. Saunders Company, 218 W. Washington Sq., Philadelphia 5; 7 Grape St., Shaftesbury Ave., and London, W.C. 2, 1952.

A PATHOLOGY OF THE EYE. By Eugene Wolff, M.B., B.S. (Lond.), F.R.C.S. (Eng.), ophthalmic surgeon, Royal Northern Hospital; surgeon, and late pathologist and lecturer in anatomy to the Westminster Branch of the Moorfields, Westminster and Central Eye Hospital; late demonstrator of anatomy, University College; late chief clinical assistant, Royal London Ophthalmic Hospital (Moorfields) and Ophthalmic Registrar, University College Hospital. Pp. 364, with 318 illustrations and 5 color plates. Price $9.50. The Blakiston Company (Division of Doubleday & Company, Inc.), 1012 Walnut St., Philadelphia 5, 1951.

The appearance of the third edition of this well-written and well-illustrated volume attests to its popularity. New illustrations have been added, while revisions in the text include particularly complete discussions of the origin of melanoma, the histogenesis of retinoblastoma, and the pathogenesis of Coats' disease and retrolental fibroplasia.

To the general pathologist who is often unfamiliar with the anatomy and physiology of the eye—not to mention its pathologic states and the terminology applied thereto—this volume should be of inestimable help.

PRESCRIPTION FOR MEDICAL WRITING: A USEFUL GUIDE TO PRINCIPLES AND PRACTICE OF EFFECTIVE SCIENTIFIC WRITING AND ILLUSTRATION. By Edwin P. Jordan, M.D., and Willard C. Shepard. Pp. 112, with 26 illustrations. Price $2.50. W. B. Saunders Company, 218 W. Washington Sq., Philadelphia 5; 7 Grape St., Shaftesbury Ave., London, W.C. 2, 1952.

This book, comprised of 10 chapters, covers three phases of the preparation of medical papers —the actual writing, the illustrating, and the use of statistics. To save the reader's time, the authors have written in simple, sometimes humorous, and quickly read style.

The section on illustrations stresses important points of which journal editors have found a vast number of contributors apparently unaware. It is of special value.

A detailed table of contents and index, plus appendices covering style of reference lists, common abbreviations, proofreaders' marks, tables of weights, measures, and temperatures, and probability scales, add to the book's usefulness.

Editors of medical journals might hope that this book will be read by every potential author of a scientific paper and that it will be consulted frequently when specific questions concerning any phase of medical writing arise.

AN ATLAS OF NORMAL RADIOGRAPHIC ANATOMY. By Isadore Meschan, M.A., M.D., professor and head of the Department of Radiology, University of Arkansas School of Medicine. With the assistance of R. M. F. Farrer-Meschan, M.B., B.S. (Melbourne, Australia). Pp. 593, with 1,044 illustrations on 362 figures. Price $15. W. B. Saunders Company, 218 W. Washington Sq., Philadelphia 5; 7 Grape St., Shaftesbury Ave., London, W.C. 2, 1951.

This new atlas provides a succinct description of fundamental anatomy in its application to clinical radiology. The numerous correlated plates of line drawings and roentgenograms serve to demonstrate the routine methods of radiographic projection, the orientation of the film so obtained, and the accurate identification of the anatomic parts visualized on these films. The book will make its primary appeal to the student of radiology, but the anatomist and the pathologist can gain much from it. The first chapter is a clear, concise, and well-illustrated account of the basic physical background for radiologic examination. It will bear reading by any physician who desires an acquaintance with the elementary principles of radiographic theory and technic. The pathologist will find of particular interest the data on changes in bone growth and development, the more common variations from normal bone structure as revealed in the roentgenogram, and the brief discussion of the use of contrast materials for demonstration of the coronary circulation at autopsy.

This book will serve as an accurate guide to normal and variational radiographic anatomy, and can be recommended as a reference source for those teachers of pathology who employ the adjunct aid of radiology in correlating morbid gross structure with the radiographic appearances of disease in the living subject.

PATHOLOGY OF THE FETUS AND THE NEWBORN. By Edith L. Potter, M.D., Ph.D., associate professor of pathology, Department of Obstetrics and Gynecology, University of Chicago; pathologist, Chicago Lying-In Hospital; chief pathologist, Chicago Department of Health. Pp. 574, with 601 illustrations. Price $19. The Year Book Publishers, Inc., 200 E. Illinois St., Chicago 11, 1952.

The title of this book indicates the field of study in which the author is a pioneer and a recognized authority. The concise and accurate presentation of the pathologic aspects of fetal disorders is a reflection of the author's competence and thorough understanding of the subject. The book is replete with illustrations, which are among the best ever published in this field. Genuine care has been taken in the selection and reproduction of each of them. The book should appeal particularly to the classic pathologist because of the emphasis upon morbid anatomy and developmental anomalies. It is recommended that all pediatricians and obstetricians should spend some spare time in learning what the author has written and illustrated so well. No book can contain everything about any subject of broad medical interest, but it is hoped that the author will find space in future editions to increase the breadth of her presentation. This field of study has not developed fully, and it is within the province of the pathologist to point out those areas in which other medical scientists have made important contributions. Furthermore, there are many areas in which bacteriologists, physiologists, chemists, and geneticists could profitably make additional original investigations. The pathologist has been and will probably continue to be the principal stimulus to those in allied sciences who are familiar with important methods but unfamiliar with problems of disease which might be solved by use of their methods. Whatever changes of this nature the author may choose to make in future editions, the book in its present form is the best available monograph on the pathologic changes which may occur in the human fetus from the moment of conception until the third natural month of extrauterine life.

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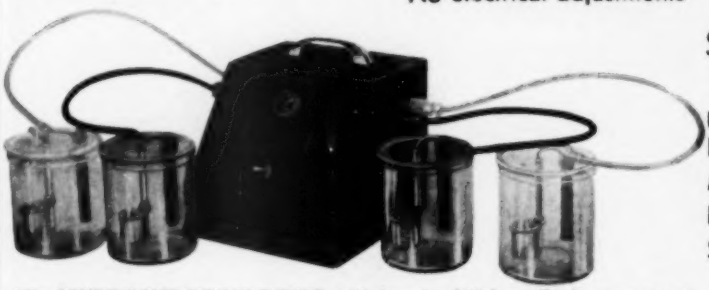

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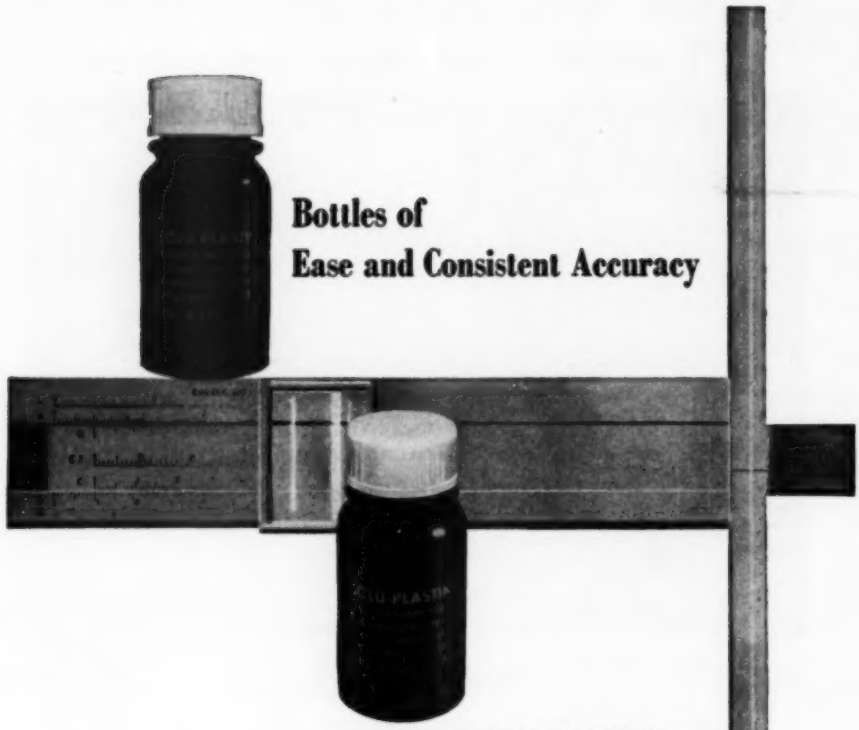

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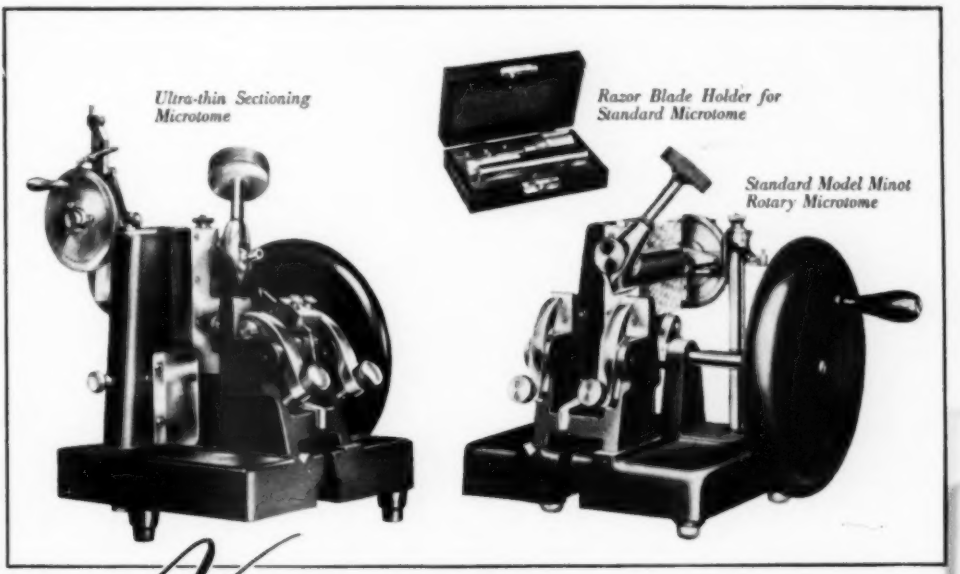


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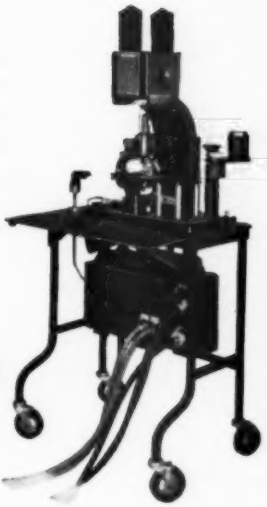

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